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Sequential Reuse of Spent Coffee Grounds in Greek Coffee Preparation: Changes in Ground and Beverage Properties Across Brewing Cycles and Consumer Attitudes in Greece

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Abstract

Spent coffee grounds retain potentially valuable constituents after brewing and may therefore be suitable for reuse in subsequent coffee preparations. This study characterized the grounds exploited in three brewing cycles and the resulting beverages from two commercial Greek coffee blends containing 70% Arabica/30% Robusta (w/w) and 80% Arabica/20% Robusta (w/w). Total phenolic content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity were determined in the original grounds, the grounds used in subsequent preparations, and the resulting beverages. Ultrasound-assisted extraction was optimized by response surface methodology, with DPPH radical-scavenging capacity as the response. Extraction was used to prepare solid samples for analysis, whereas beverages were analyzed directly. Methanol concentration and extraction time significantly affected the response, whereas temperature did not. At the optimum (22% v/v methanol, 61 min, 47 °C), the predicted and experimental responses were 16,614 mg/kg and 16,039 mg/kg ± 432 mg/kg, respectively. Relative to the initial grounds, total phenolic content and DPPH capacity were 13.4% and 12.8% in Cycle 2 and 3.9% and 3.4% in Cycle 3 for the 70% (w/w) Arabica blend, compared with 25.9% and 10.2% in Cycle 2 and 10.4% and 9.2% in Cycle 3 for the 80% (w/w) Arabica blend. Turbidity and sedimentation showed cycle- and blend-dependent behavior, while microscopic examination revealed heterogeneous particle morphology in the grounds. An anonymous questionnaire conducted in Greece explored consumer perceptions of coffee-residue reuse and stated willingness to consider related applications. The findings characterize changes in the grounds and beverages during repeated brewing and provide insight into the potential and limitations of coffee-ground reuse.

Keywords: Greek coffee; spent coffee grounds; sequential reuse; circular bioeconomy; ultrasound-assisted extraction; radical-scavenging capacity; response surface methodology; consumer perceptions

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1. Introduction

Biomass generated by agricultural and food-processing activities represents a potential resource for producing energy, materials, and value-added compounds. Its valorization can involve thermochemical processes, including pyrolysis, gasification, liquefaction, and combustion, which convert biomass into products such as biochar, bio-oil, syngas, biofuels,

and heat [1–3]. Biomass can also be valorized through biological processing, which enables the production of useful chemicals, materials, and enzymes from renewable feedstocks [4,5]. Alternatively, extraction-based approaches can recover valuable constituents from agri-food byproducts, including phenolic compounds and other potentially bioactive substances, depending on the composition of the biomass and the extraction method used [6,7]. The choice of processing route depends on the composition of the biomass and the intended application. Within this context, spent coffee grounds are an abundant residual material with potential for several valorization strategies.

Coffee is one of the most widely consumed beverages worldwide, with global production estimated at 177.5 million bags in 2024/25 [8]. Coffee processing and brewing generate substantial quantities of residual material, including spent coffee grounds, which have been estimated at approximately 6.7 million tonnes worldwide [9]. These residues retain a diverse range of potentially valuable constituents, including phenolic compounds, proteins, lipids, polysaccharides, amino acids, alkaloids, minerals, lignin, and melanoidins [10]. Their chemical composition and associated bioactive properties have therefore attracted increasing interest in valorization within circular bioeconomy approaches [9,10]. The characteristics of spent coffee grounds can, however, vary with the type of coffee beans, roasting conditions, and extraction process [10]. One preparation of particular interest is Greek coffee, a boiled beverage in which roasted ground beans are heated together with water, leaving a solid residue at the bottom of the serving cup that is not consumed [11].

Greek coffee is commonly prepared from blends of *Coffea arabica* and *Coffea robusta*, two commercially important coffee species with distinct chemical compositions. These variations include compounds such as caffeine, chlorogenic acids, lipids, amino acids, oligosaccharides, polysaccharides, and minerals, while roasting can substantially affect bioactive compounds and other quality-related characteristics [11,12]. The brewing process can further modify the physicochemical and sensory properties of the resulting beverage, including pH, turbidity, total and filtered solids, fatty acid content, and aroma [13].

The properties of spent coffee grounds change progressively with successive brewing cycles. Recent studies show that while this sequential use alters their bioactive composition, measurable amounts of constituents, such as phenolic acids and flavonoids, still remain in the final residue [14,15]. Furthermore, repeated brewing has been associated with structural modifications, including increased moisture retention and porosity [14]. Consequently, these combined chemical and structural shifts yield a dynamic residual matrix that evolves continuously with each subsequent reuse cycle.

The extraction of valuable compounds from spent coffee grounds depends on factors such as solvent composition and extraction conditions, which influence the recovery of target compounds [16]. Ultrasound-assisted extraction has been investigated for recovering bioactive compounds from coffee waste, particularly phenolic and flavonoid compounds [17]. Its operating conditions have also been optimized using response surface methodology (RSM) for the extraction of oil from spent coffee grounds [18]. RSM has further been applied to optimize ultrasound-assisted extraction from different plant-based materials, with studies evaluating variables such as solvent-to-solid ratio, solvent composition, temperature, and extraction time, and responses including free radical-scavenging activity and total phenolic content [19–21]. These studies illustrate how RSM can be adapted to different extraction variables and analytical objectives. In contrast to the previous optimization of oil recovery from spent coffee grounds [18], the present study used free radical-scavenging capacity as the optimization response and subsequently determined both free radical-scavenging capacity and total phenolic content in the extracts. Free radical-scavenging capacity provides an in vitro measure of radical-scavenging activity, although its interpretation depends on the assay used [22]. The optimized extraction conditions were then applied to the analytical

assessment of grounds generated during sequential Greek coffee brewing, complementing the characterization of the corresponding beverages.

The valorization of coffee residues for food applications depends heavily on consumer perceptions and acceptance. Studies show that consumers generally view upcycled foods positively, associating them with innovation, sustainability, and waste reduction [23,24]. However, positive attitudes do not automatically translate into buying behavior, as limited awareness remains a major barrier [23]. Negative associations, such as scepticism, dislike, and perceptions of disgust, can occur [24]. Acceptance may further depend on perceived risk and value, price, trust in regulations, and innovation-related factors [25]. These findings indicate that consumer attitudes and responses are relevant alongside the technical assessment of materials considered for upcycled or circular applications.

Despite growing interest in the valorization of spent coffee grounds, the effects of successive preparation cycles on traditional Greek coffee and the grounds remaining after brewing have not been fully explored. Previous research has characterized the bioactive composition of spent coffee grounds obtained using different brewing methods [26] or examined their valorization in applications such as textile wastewater treatment and bacterial cellulose production [27,28], without investigating their reuse in coffee preparation. More recently, the impact of repeated brewing on the bioactive composition, antioxidant capacity, and physicochemical characteristics of spent grounds has been examined, including the influence of coffee variety and roasting level [14,15]. However, these studies focused on the spent grounds, leaving the characteristics of the resulting beverages largely unaddressed. In particular, changes in traditional Greek coffee beverages across repeated brewing cycles and their relationship with the characteristics of the grounds reused in subsequent preparations remain poorly understood. Consumer perceptions of coffee-residue reuse in the context of Greek coffee also warrant further attention. Addressing these gaps would clarify changes in both the beverages and the grounds during repeated preparation and provide preliminary insight into consumers' stated willingness to consider applications involving coffee-residue reuse.

This study investigated two commercial Greek coffee blends over three brewing cycles, examining changes in the residual grounds and the properties of the resulting beverages, alongside an exploratory survey of Greek consumers' awareness, perceptions, and willingness to consider coffee-residue reuse applications. The study focused on characterizing these materials rather than producing a separate extract or a treated residue. The blends contained 70% (w/w) and 80% (w/w) Arabica coffee, respectively, with the remaining proportions consisting of Robusta coffee. Free radical-scavenging capacity and total phenolic content were determined in the original grounds, the spent grounds reused in subsequent preparations, and the resulting beverages. Ultrasound-assisted extraction was optimized and employed to prepare the solid samples for these analytical measurements, whereas the liquid samples were assessed directly. Beverage pH, turbidity, and sedimentation were measured, while optical microscopy was used to qualitatively assess the microstructure of the coffee grounds used in each brewing cycle. An anonymous questionnaire explored Greek consumers' awareness of coffee components and residue reuse, their sustainability attitudes, and their willingness to consume Greek coffee produced using technologies involving coffee-residue utilization.

2. Materials and Methods

2.1. Coffee Samples

Two commercially available ground Greek coffee products were purchased from Coffee Island (Thessaloniki, Greece): Greek Coffee Traditional (in bulk), corresponding to a 70% Arabica/30% Robusta (w/w) blend with a medium roast, and Greek Coffee Special Selection

(in bulk), corresponding to an 80% Arabica/20% Robusta (*w*/*w*) blend with a light–medium roast. Both samples were used as received, without additional pretreatment before the first brewing cycle. The two samples were selected to investigate changes in the chemical and physical characteristics of the coffee material during sequential reuse across two commercial products with different blend compositions and roasting degrees. As the Arabica/Robusta ratio and roasting degree differed simultaneously between the samples, their individual contributions to the observed differences could not be distinguished. The results therefore reflect the combined characteristics of each coffee product rather than the independent effects of blend composition or roasting degree. The samples were subsequently subjected to a typical Greek coffee preparation procedure, as described in Section 2.4.

2.2. Chemicals and Reagents

Methanol (≥99.8%) was supplied by Chem–Lab NV (Zedelgem, Belgium), whereas ethanol (≥99.8%) and acetone (≥99.8%) were received from Fisher Scientific (Loughborough, UK). Folin–Ciocalteu reagent was obtained from Sordalab (Étampes, France). Anhydrous gallic acid (≥99%) was purchased from Glentham Life Sciences (Corsham, UK). Anhydrous sodium carbonate (≥99.5%) was a product of Labkem (Barcelona, Spain). 1,1-Diphenyl-2-picrylhydrazyl (DPPH; >97%) was supplied by TCI Europe N.V. (Zwijndrecht, Belgium). Drinking water was used for Greek coffee brewing. Deionized water was produced with a Zalion 600 ion-exchange water purification system (Ionel S.A., Athens, Greece) and used throughout the analytical procedures.

2.3. Instrumentation

The experimental procedures involved a range of laboratory equipment. Spectrophotometric measurements were obtained with a UV–1600PC UV–Vis spectrophotometer (VWR, Radnor, PA, USA). Ultrasound-assisted extraction was carried out in a JPS10A ultrasonic bath (Vevor, Frankfurt, Germany; 2 L capacity, 40 kHz ultrasonic frequency, 60 W ultrasonic power, and 100 W heating power). Sample heating and drying were achieved with an MSH-300 magnetic stirrer equipped with a hot plate (Biosan, Riga, Latvia) and a natural-convection oven (JeioTech, Daejeon, Republic of Korea), respectively. Sample homogenization was carried out with a Vortex V-1 Plus mixer (Biosan, Riga, Latvia), whereas mass measurements were obtained on an ACN220G analytical balance (AXIS Sp. z o.o., Gdańsk, Poland). pH values were determined with an MW151 MAX pH/ORP/Temp logging bench meter (Milwaukee Instruments, Szeged, Hungary). Microscopic observations were made with a TFM 201/301 + Infinity microscope, and digital images were captured with a MikrOkular Full HD Eyepiece Digital Camera. Image acquisition and processing were conducted with CamLabLite software, version 2.1.29083.20250729. All three products were supplied by Bresser (Rhede, Germany). Coffee samples were prepared using a professional coffee preparation kit (El Greko, Athens, Greece), consisting of a traditional *briki* and a dedicated gas burner (1500 W) with adjustable flame intensity.

2.4. Greek Coffee Brewing and Spent Coffee Grounds Reuse

The sequential Greek coffee brewing and spent coffee grounds reuse procedure is summarized in Figure 1. For each preparation in all three brewing cycles, 4.60 g of solid material and 60 mL of drinking water were introduced into a traditional Greek coffee pot (*briki*), corresponding to a nominal solid-to-water ratio of 1:13 (*w*/*v*). Commercial ground coffee was used in Cycle 1, whereas dried spent coffee grounds recovered from the preceding cycle were used in Cycles 2 and 3. The mixture was heated over a gas burner under gentle stirring until the foam (*kaimaki*) reached the upper rim of the *briki*, typically within 2–3 min, marking the endpoint of the process. The prepared beverage was subsequently transferred

to a cup and allowed to stand for approximately 2 min. The liquid phase was then separated from the spent coffee grounds using a Büchner funnel equipped with a filter with a pore size of 8–12 µm. The resulting filtrate was collected for subsequent physicochemical analyses, while the recovered solid fraction was retained for drying, analysis, and reuse in the next brewing cycle.

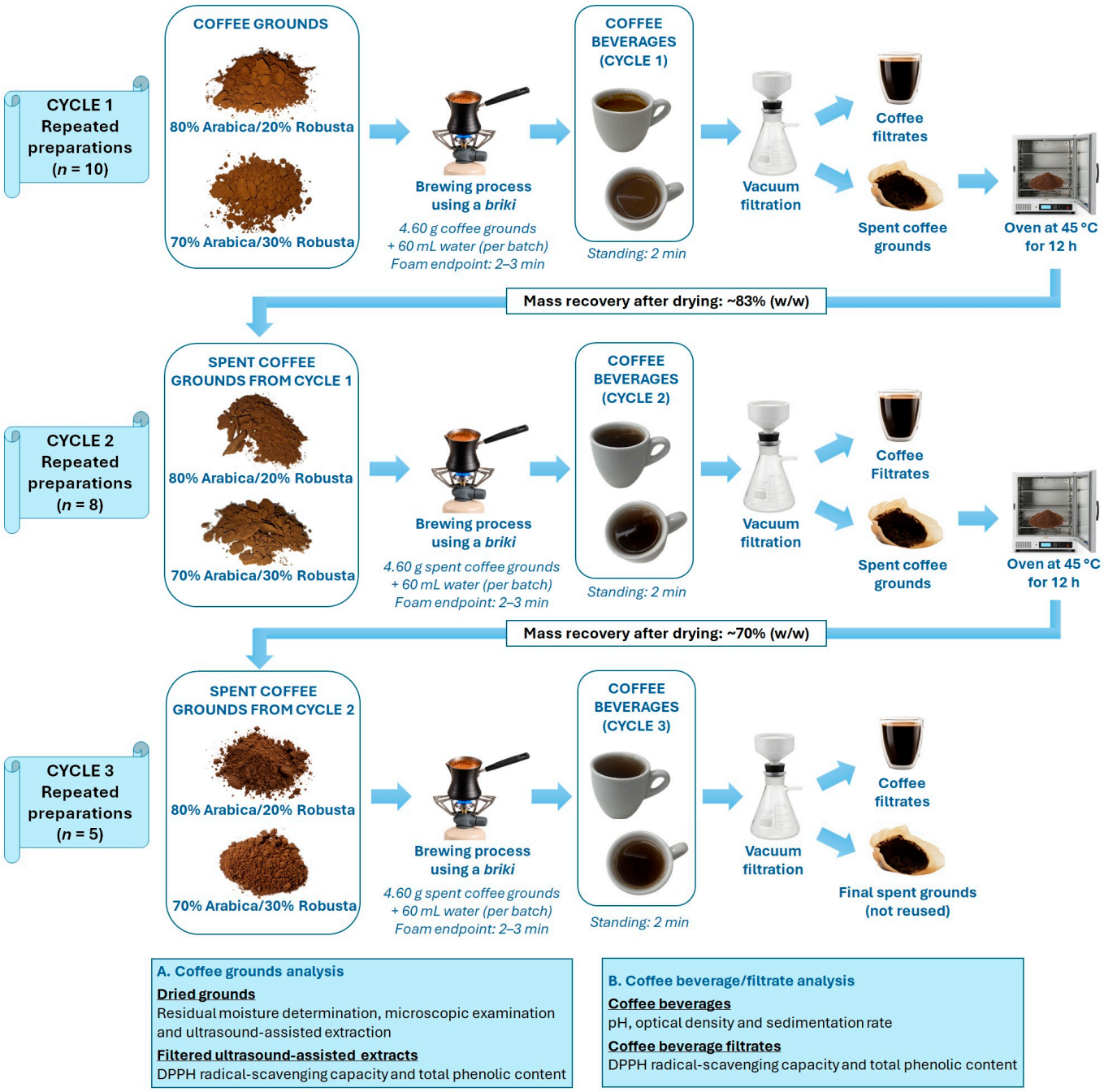


Figure 1. Schematic representation of the sequential Greek coffee brewing process over three consecutive brewing cycles, including beverage preparation, vacuum filtration, drying, and reuse of spent coffee grounds. The coffee grounds and beverages shown are the actual materials obtained experimentally. For each coffee blend, repeated preparations were combined separately for each cycle to obtain sufficient material for subsequent reuse and analysis. The numbers of repeated preparations were 10, 8, and 5 for Cycles 1, 2, and 3, respectively. The spent coffee grounds recovered after Cycle 3 were not reused.

For clarity, Cycle 1 refers to the initial brewing of commercial ground coffee, Cycle 2 to the brewing of the spent grounds recovered from Cycle 1, and Cycle 3 to the brewing of the spent grounds recovered from Cycle 2. The term "brewing cycle" identifies the brewing step, whereas the terms "original ground coffee," "spent coffee grounds after Cycle X," and "coffee beverage obtained in Cycle X" identify the specific material or sample analyzed.

Following filtration, the spent coffee grounds were transferred to a natural-convection oven and dried at 45 °C for approximately 12 h before weighing and reuse. For each coffee blend, the brewing procedure was repeated 10 times in Cycle 1, 8 times in Cycle 2, and 5 times in Cycle 3. The recovered grounds from the respective preparations were combined separately for each blend and cycle before drying. In Cycle 1, 46.0 g of commercial ground coffee yielded 38.2 g of spent grounds after drying, corresponding to a mass recovery of 83.0%. In Cycle 2, 36.8 g of previously dried spent grounds yielded 25.8 g of residue after the same brewing and drying procedure, corresponding to a mass recovery of 70.1%. These values represent mass recovery following the specified drying treatment. The grounds recovered after Cycle 3 were not reused.

2.5. Determination of Moisture Content

Coffee grounds obtained from all three brewing cycles were spread in a thin, uniform layer of approximately 2 mm thickness and dried in an oven at 45 °C for 12 h. The residual moisture content of the spent coffee grounds after drying at 45 °C for 12 h was determined gravimetrically by weighing the samples before and after drying at 103 °C to constant mass. Moisture content was expressed on a wet basis (w/w) and calculated according to Equation (1):

$$\text{Moisture } (\%) = \left(\frac{m_i - m_d}{m_i} \right) \times 100\% \quad (1)$$

where m_i is the sample mass before drying at 103 °C and m_d is the sample mass after drying to constant mass.

2.6. Preliminary Solvent Screening

A preliminary solvent-screening experiment was conducted to compare the radical-scavenging responses obtained using different extraction solvent systems. For each extraction, 0.25 g of ground coffee material was mixed with 15 mL of extraction solvent. Water, methanol, 50% aqueous methanol (v/v), ethanol, 50% aqueous ethanol (v/v), acetone, and 50% aqueous acetone (v/v) were evaluated under identical ultrasound-assisted extraction conditions (30 °C for 30 min). Following extraction, the suspensions were filtered through an 8–12 µm filter, and the resulting extracts were assessed using the DPPH assay. The responses obtained with the different solvent systems were compared to select the solvent system for subsequent RSM optimization.

2.7. Ultrasound-Assisted Extraction

For ultrasound-assisted extraction, 0.25 g of either ground coffee powder or dried spent coffee grounds from the corresponding brewing cycle was transferred to a 50 mL Falcon tube. The material was mixed with 15 mL of methanol–water extraction solvent. The tubes were placed in an ultrasonic bath operating at 40 kHz and 60 W. The extraction conditions were optimized using RSM with the 80% Arabica/20% Robusta (w/w) ground coffee sample, according to the experimental design. The optimized conditions (22% v/v methanol, 61 min, and 47 °C) were then applied to the original ground coffee samples and the corresponding dried spent coffee grounds from all three brewing cycles for both coffee blends.

After sonication, the suspensions were filtered through a Büchner funnel fitted with an 8–12 μm filter. The resulting extracts were used to determine DPPH radical-scavenging capacity (Section 2.9) and total phenolic content (Section 2.10). Coffee beverages obtained after each brewing cycle were analyzed directly, without an additional extraction step. The ultrasound-assisted extraction was performed exclusively as an analytical sample-preparation step to determine the chemical characteristics of the solid samples. The resulting extracts were not intended as products and did not constitute part of the valorization approach investigated in this study.

2.8. Experimental Design and Optimization of the Extraction Procedure

RSM was employed to investigate the effects of extraction conditions on DPPH radical-scavenging capacity and to determine the combination of variables predicted to maximize the response. The optimization study was performed using the 80% Arabica/20% Robusta (w/w) coffee powder. A face-centered central composite design was generated using Minitab® statistical software (version 22.4, State College, PA, USA). Based on the preliminary solvent-screening experiment, methanol was used as the organic solvent in the optimization study. Three independent variables were investigated: methanol concentration (X_1, 0–100%, v/v), extraction time (X_2, 10–90 min), and extraction temperature (X_3, 30–60 °C). The experiments were conducted in randomized order. The independent variables and their coded and actual levels are presented in Table 1.

Table 1. Coded and actual levels of the independent variables used in the response surface methodology experimental design for the optimization of Greek coffee extraction.

Factors	Variables	Levels		
		Coded values		
		−1	0	+1
		Actual values		
Methanol concentration (%, v/v)	X_1	0	50	100
Extraction time (min)	X_2	10	50	90
Extraction temperature (°C)	X_3	30	45	60

The response variable was DPPH radical-scavenging capacity (mg/kg). The experimental data were fitted to a full second-order (quadratic) polynomial model, including linear, quadratic, and two-factor interaction terms, according to Equation (2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

where Y_{DPPH} is the predicted response; X_1, X_2, and X_3 are the independent variables; and β_0, β_1, β_2, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, and β_{23} are the estimated regression coefficients, with β_0 representing the model intercept.

The DPPH radical-scavenging capacity (mg/kg) was selected as the optimization criterion because it provided a practical analytical response for comparing the recovery of radical-scavenging constituents under the investigated extraction conditions. Numerical optimization was performed to maximize the predicted response within the experimental ranges of the three independent variables.

2.9. Determination of DPPH Radical-Scavenging Capacity

The free-radical scavenging capacity of ground coffee powders, spent coffee grounds, and coffee filtrates was evaluated using the DPPH assay [20]. For ground coffee powder and spent coffee grounds, the samples were extracted prior to analysis, whereas coffee

beverages were analyzed directly without an extraction step. An appropriate aliquot of each sample was added to 3 mL of freshly prepared 0.2 mM DPPH solution in methanol. Sample aliquot volumes of 50 µL, 100 µL, or 500 µL were selected according to the observed radical-scavenging response. Larger aliquots were used for samples with lower activity, whereas smaller aliquots and sample dilution were used for samples with higher activity, where necessary. The resulting mixtures were incubated in the dark at ambient temperature for 30 min. The absorbance was subsequently measured at 517 nm using the UV–Vis spectrophotometer.

For each sample, a corresponding sample blank was prepared by replacing the DPPH solution with pure methanol to account for background absorbance arising from the sample matrix. A control solution was prepared by replacing the sample aliquot with the corresponding solvent and applying the same experimental conditions. The DPPH radical-scavenging capacity was calculated according to Equation (3):

$$\text{Radical} - \text{scavenging capacity } (\%) = \left(\frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \times 100\% \quad (3)$$

where A_{Sample} and A_{Control} correspond to the absorbance values of the sample and control solutions, respectively.

Gallic acid calibration curves were constructed using standard aliquots of 50, 100, and 500 µL under the same DPPH assay conditions as the samples. The calibration ranges were 10–200 mg/L for the 50 µL aliquot, 5–100 mg/L for the 100 µL aliquot, and 1–20 mg/L for the 500 µL aliquot. The corresponding linear regression equations were $y = 0.2841x + 6.2572$, $y = 0.5398x + 7.8766$, and $y = 2.8393x + 6.4102$, respectively, with coefficients of determination (R^2) ranging from 0.995 to 0.998, where y represents DPPH radical-scavenging activity (%) and x represents the gallic acid concentration (mg/L). Sample responses were converted to gallic acid-equivalent concentrations using the calibration equation corresponding to the aliquot volume used. For ground coffee powder and spent coffee grounds, the calculated amounts were normalized to the initial sample mass and expressed as mg/kg, accounting for the extraction volume and applicable dilution factors. Coffee beverage concentrations were expressed as mg/L, with dilution factors applied where necessary.

For the optimization experiments, DPPH radical-scavenging capacity was used as the dependent variable. The assay provided a measure of free-radical scavenging under the specified experimental conditions and was not intended to represent physiological antioxidant activity. Following model optimization, the predicted optimum conditions were experimentally validated by performing an independent DPPH assay under the optimized extraction conditions. The validation experiment was conducted using the same analytical procedure described above.

2.10. Determination of Total Phenolic Content

The total phenolic content of ground coffee powders, spent coffee grounds, and coffee filtrates was determined using the Folin–Ciocalteu colorimetric assay [20]. Prior to analysis, the samples were appropriately diluted, when required, to obtain absorbance values within the working range of the calibration curve. For each determination, 200 µL of the diluted sample was transferred into a 10 mL volumetric flask and mixed with 5 mL of deionized water. Subsequently, 500 µL of Folin–Ciocalteu reagent was added, and the mixture was kept in the dark for 3 min. Then, 1 mL of 37% (w/v) aqueous sodium carbonate solution was added, and the volume was adjusted to 10 mL with deionized water. The reaction mixture was incubated in the dark for 1 h, after which the absorbance was measured at 725 nm using the UV–Vis spectrophotometer. A reagent blank was prepared by replacing the sample aliquot with the corresponding solvent or, for beverage samples, the appropriate blank medium. The concentration of Folin–Ciocalteu-reactive compounds was determined

using a gallic acid calibration curve and expressed as gallic acid equivalents. For ground coffee powder and spent coffee grounds, the results were normalized to the initial mass of solid material subjected to extraction (0.25 g extracted with 15 mL of solvent) and expressed as mg/kg dry weight. For coffee beverages, the total phenolic content was expressed as mg/L of beverage.

2.11. pH Measurement

The pH of the brewed coffee beverages was determined after each brewing cycle for both coffee samples. Before each series of determinations, the pH meter was calibrated using standard buffer solutions at pH 4.00 and 7.00. As the freshly prepared coffee beverages were initially warm, the samples were allowed to cool to approximately room temperature. Measurements were performed directly in the coffee serving cup under conditions representative of the beverage as served, and the value was recorded once a stable reading was obtained.

2.12. Evaluation of Suspension Stability

The physical stability of the prepared Greek coffee beverages was evaluated by monitoring the optical density (OD) at 700 nm as an indicator of suspension turbidity, following a spectrophotometric approach reported for food dispersions [29]. Sedimentation behavior was characterized by the decrease in optical density over a defined settling period, following a previously described sedimentation approach [30].

The coffee beverages were prepared according to the standardized brewing procedure described in Section 2.4 and gently stirred to obtain a homogeneous suspension. Subsequently, 3 mL of the beverage was carefully withdrawn from the middle layer, avoiding the surface foam and settled material at the bottom. Drinking water was used as the spectrophotometric blank. The initial optical density was recorded at time zero (OD_0), followed by measurements at 1-min intervals over a total observation period of 20 min. During the measurements, the samples were maintained at room temperature under static conditions to avoid disturbance of the natural sedimentation process. Changes in optical density over time were used to characterize the sedimentation behavior and turbidity of the coffee beverages.

The sedimentation rate (R_S) was calculated from the decrease in optical density over the settling period according to Equation (4):

$$R_S = \frac{OD_0 - OD_t}{t} \quad (4)$$

where OD_0 is the initial optical density, OD_t is the optical density measured at the end of the 20-min settling period, and t is the elapsed time (20 min). The R_S was expressed in min^{-1}.

The OD_{700} measurements were recorded directly, without sample dilution. According to the manufacturer's specifications, the UV-1600PC spectrophotometer has a photometric range of −0.3 to 3.0 absorbance units. Although most measurements were within this specified range, the highest value ($OD_0 = 3.040$) slightly exceeded its upper limit. Furthermore, the linearity of the instrument's response at absorbance values above approximately 2 units was not independently verified. Therefore, the high OD_0 values, particularly the value exceeding the specified photometric range, should be interpreted with caution, as their accuracy may be affected by limitations in the instrument's photometric response.

2.13. Microscopic Observation and Morphological Characterization

The morphology of the ground coffees and spent coffee grounds was evaluated using optical microscopy. Approximately 2.5 mg of ground coffee or spent coffee grounds were suspended in 1 mL of deionized water and gently agitated to disperse the material in the aqueous medium. Subsequently, 20–30 µL of the prepared suspension was transferred onto

a clean microscope slide and covered with a coverslip. Care was taken to minimize air bubbles and excessive particle overlap.

Microscopic observations were performed using 10× and 40× objective magnifications. Representative fields were captured using a MikrOkular Full HD digital eyepiece camera and processed using CamLabLite software (version 2.1.29083.20250729; Bresser, Rhede, Germany). The micrographs were examined qualitatively to characterize the apparent morphology of the ground coffees and spent coffee grounds, focusing on particle shape, irregularity, apparent size, color, and the presence of closely associated particles or larger structures. Representative micrographs obtained at both magnifications were selected for presentation.

2.14. Summary of Experimental Conditions

The main experimental conditions used for Greek coffee brewing, spent coffee grounds reuse, drying, moisture determination, and ultrasound-assisted extraction are summarized in Table 2. The brewing conditions were kept consistent across the three cycles, while the extraction conditions for the solid samples were established through preliminary solvent screening and RSM optimization. Table 2 also identifies the sample fractions subjected to the different analyses.

Table 2. Summary of the main experimental conditions used in the study.

Procedure	Experimental Conditions
Greek coffee brewing	In total, 4.60 g solid material and 60 mL drinking water per preparation; heating in a traditional *briki* until the foam reached the rim (typically 2–3 min); standing for 2 min
Filtration of beverages	Vacuum filtration using a Büchner funnel with an 8–12 µm filter
Repeated preparations	In total, 10, 8, and 5 preparations per coffee blend for Cycles 1, 2, and 3, respectively
Drying of spent grounds	Natural-convection oven at 45 °C for approximately 12 h; grounds spread in a uniform layer of approximately 2 mm
Moisture determination	Gravimetric method; drying at 103 °C to constant mass
Preliminary solvent screening	In total, 0.25 g solid sample, 15 mL solvent, 30 °C, and 30 min
Ultrasound-assisted extraction for analytical purposes	In total, 0.25 g solid sample, 15 mL methanol–water solvent; 40 kHz and 60 W
RSM-optimized analytical extraction	22% (*v*/*v*) methanol; 61 min; 47 °C
Analyses of beverage samples	pH, optical density, and sedimentation rate
Analyses of filtered ultrasound extracts	DPPH radical-scavenging capacity and total phenolic content
Microscopic examination	Qualitative characterization of original ground coffee and spent coffee grounds

2.15. Online Consumer Questionnaire

An anonymous online questionnaire was conducted as a complementary, exploratory component of the study to assess consumer habits, knowledge, and perceptions regarding Greek coffee consumption and the potential valorization of spent coffee grounds. The survey addressed consumer attitudes toward the possible applications of coffee residues and the acceptability of Greek coffee produced using technologies involving coffee-residue valorization. It was not designed to evaluate the methanolic extracts obtained in the experimental part directly, but rather to investigate consumer perceptions of potential valorization applications. The questionnaire was developed in accordance with the objectives of the

study. Participants were recruited using convenience sampling, with the questionnaire link distributed through social media and personal contacts. Data were collected from 2 to 28 August 2026.

The questionnaire was administered electronically using Microsoft Forms and was completed by 82 adults aged 18 years or older. Participation was voluntary, anonymous, and completion required approximately 5–7 min. The questionnaire comprised four sections addressing demographic characteristics, Greek coffee consumption habits, knowledge regarding coffee components and spent coffee grounds, and perceptions related to health, sustainability, and potential applications of spent coffee grounds. It was developed specifically for the objectives of this exploratory study and was not presented as a previously validated psychometric instrument. The complete questionnaire, including all questions and response options, is provided as Supplementary Material.

The research protocol received institutional low-risk ethical approval from New York College (Greece) on 23 July 2026. The ethics approval documentation was provided confidentially to the journal. The study was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki [31], the General Data Protection Regulation (EU) 2016/679 [32], and the institutional research ethics standards of New York College. No directly identifiable personal information was collected, and responses were analyzed in aggregated form.

2.16. Statistical Analysis

RSM analysis was carried out with Minitab® statistical software (version 22.4, State College, PA, USA). Analysis of variance (ANOVA) was performed to assess the significance of the fitted second-order polynomial model and its individual terms. Model adequacy was evaluated based on the coefficient of determination (R^2), adjusted R^2, predicted R^2, and the lack-of-fit test. Numerical optimization was applied to identify the extraction conditions predicted to maximize DPPH radical-scavenging capacity. A p-value < 0.05 was considered statistically significant.

The analytical measurements and questionnaire data were analyzed using JASP software (version 0.98.1, Amsterdam, The Netherlands). Analytical results were expressed as mean ± standard deviation ($n = 3$). For ground coffee and spent coffee grounds, the three replicates represent separate analytical extractions of material obtained from multiple brewing preparations combined for each blend and brewing cycle. Thus, these replicates characterize analytical variability rather than variability between independent brewing experiments. As the brewing cycles were sequentially derived, comparisons between blends and brewing cycles were interpreted descriptively.

For the preliminary solvent-screening experiment, differences among solvent systems were assessed using one-way ANOVA, followed by Tukey's honestly significant difference (HSD) post hoc test when the overall ANOVA was significant. Homogeneity of variances was assessed using Levene's test, which indicated no significant evidence of unequal variances in this experiment. For questionnaire data, categorical responses were summarized as frequencies and percentages. Five-point ordinal attitude measures were summarized using the median, interquartile range (IQR), and minimum–maximum values. Associations among ordinal attitude measures were assessed using Spearman's rank correlation coefficient (ρ). Pearson's correlation analysis was also used to assess the association between DPPH radical-scavenging capacity and total phenolic content determined by the Folin–Ciocalteu assay, separately for the solid and beverage fractions. A p-value < 0.05 was considered statistically significant.

3. Results

This study aimed to characterize changes in the physicochemical properties and bioactive-related characteristics of two commercial Greek coffee blends and their corresponding spent coffee grounds during three successive brewing cycles. Specifically, the study evaluated DPPH radical-scavenging capacity and total phenolic content in the solid and beverage fractions, examined changes in beverage pH, optical density, and sedimentation behavior, and qualitatively characterized the morphology of the spent coffee grounds by light microscopy. In addition, ultrasound-assisted extraction conditions were optimized using RSM, and consumer perceptions regarding the reuse of coffee residues were explored.

3.1. Preliminary Solvent Screening and Optimization of Ultrasound-Assisted Extraction

Preliminary solvent screening included water, methanol, 50% (v/v) aqueous methanol, ethanol, 50% (v/v) aqueous ethanol, acetone, and 50% (v/v) aqueous acetone. The screening was conducted under fixed ultrasound-assisted extraction conditions (30 °C for 30 min), and the DPPH radical-scavenging results are presented in Table 3. Water yielded the highest numerical DPPH radical-scavenging capacity (15,412.8 mg/kg ± 155.5 mg/kg), followed closely by 50% (v/v) aqueous methanol (15,293.5 mg/kg ± 136.4 mg/kg), with no statistically significant difference between these two solvent systems (Tukey's HSD test, $p = 0.938$). Methanol yielded 13,372.7 mg/kg ± 148.7 mg/kg, while the lowest value was obtained with 50% (v/v) aqueous ethanol (12,728.6 mg/kg ± 120.2 mg/kg). The differences in DPPH radical-scavenging capacity among the seven solvent systems were statistically significant (one-way ANOVA, $F(6,14) = 165.3$, $p < 0.001$). Levene's test indicated homogeneity of variances ($p = 0.955$). Tukey's HSD post hoc test was subsequently used to identify differences between individual solvent systems (Table 3).

Table 3. DPPH radical-scavenging capacity of extracts of original ground coffee obtained using different solvent systems, expressed as gallic acid equivalents [1].

Solvent System	DPPH Radical-Scavenging Capacity (mg/kg Dry Weight)
Water	15,412.8 ± 155.5 [a]
Methanol	13,372.7 ± 148.7 [bc]
Aqueous methanol (50%, v/v)	15,293.5 ± 136.4 [a]
Ethanol	13,434.8 ± 97.0 [bc]
Aqueous ethanol (50%, v/v)	12,728.6 ± 120.2 [d]
Acetone	13,222.9 ± 146.8 [b]
Aqueous acetone (50%, v/v)	13,652.3 ± 172.3 [c]

[1] Values are presented as mean ± standard deviation ($n = 3$). Different lowercase letters within the same column indicate significant differences among solvent treatments according to one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Based on the screening results, water and methanol were selected as the solvent components for the subsequent RSM study. A face-centered central composite design comprising 20 experimental runs was used to investigate the effects of methanol concentration (0–100%, v/v), extraction time, and temperature on DPPH radical-scavenging capacity, covering pure water, pure methanol, and their aqueous mixtures. The experimental conditions and corresponding DPPH radical-scavenging capacities are presented in Table 4. The observed values ranged from 10,209.7 mg/kg to 16,418.8 mg/kg. The lowest values were obtained at 100% (v/v) methanol and short extraction times, particularly at 10 min, whereas higher values were generally observed at 0–50% (v/v) methanol under several of the tested conditions. The highest experimental value (16,418.8 mg/kg) was obtained at 50% (v/v) methanol,

50 min, and 45 °C. The center-point experiments showed relatively high values, with some variation among replicates.

Table 4. DPPH radical-scavenging capacity of extracts of original ground coffee obtained under the extraction conditions investigated during RSM optimization.

Run	Methanol Concentration (%, v/v)	Extraction Time (min)	Extraction Temperature (°C)	DPPH Radical-Scavenging Capacity (mg/kg Dry Weight)
1	50	50	45	15,531.7
2	50	10	45	14,560.3
3	100	10	30	10,209.7
4	50	90	45	15,721.8
5	100	90	30	13,652.1
6	50	50	45	15,954.1
7	0	90	60	15,848.5
8	100	50	45	13,504.3
9	0	90	30	15,426.1
10	0	50	45	16,207.6
11	50	50	45	16,186.4
12	100	10	60	10,610.9
13	50	50	30	16,355.4
14	50	50	45	16,418.8
15	100	90	60	15,109.4
16	50	50	45	16,165.3
17	0	10	30	15,574.0
18	50	50	45	16,376.5
19	0	10	60	15,468.4
20	50	50	60	15,151.6

The analysis of variance presented in Table 5 showed that the fitted quadratic model was statistically significant ($F = 28.08$, $p < 0.001$). Among the linear terms, methanol concentration and extraction time had a strong effect on DPPH radical-scavenging capacity ($p < 0.001$ for both), whereas temperature was not significant ($p = 0.533$). The quadratic terms for methanol concentration and extraction time were also significant ($p = 0.004$ and $p = 0.019$, respectively), indicating curvature in the response surface. In addition, the methanol concentration × extraction time interaction was significant ($p < 0.001$), while the interactions involving temperature were not significant ($p = 0.279$ and $p = 0.267$). These findings indicate that methanol concentration and extraction time were the main variables shaping the response, with their combined effect contributing to the observed surface curvature. The full quadratic model was retained to describe the response surface across the experimental domain investigated, including the linear, quadratic, and interaction terms. However, the non-significant temperature-related terms indicate that their individual effects were not statistically established under the conditions investigated, and the resulting optimum should therefore be interpreted with caution.

The solvent-screening and RSM experiments were conducted under different extraction conditions. Solvent screening was performed at 30 °C for 30 min, whereas the RSM design varied methanol concentration, extraction time, and temperature. Therefore, the results obtained using 100% methanol in the two experiments are not directly comparable, and the observed differences should be interpreted in the context of the different extraction conditions.

The fitted model explained 96.17% (R^2) of the variability in DPPH radical-scavenging capacity, with an adjusted R^2 of 92.73% and a predicted R^2 of 75.71%. These results indicate a good fit to the experimental data, although the lower predicted R^2 suggests more moderate predictive performance for new observations. The lack-of-fit test was not significant

($p = 0.106$), indicating no evidence of lack of fit relative to pure experimental error. To complement the ANOVA results, Table 6 presents the estimated regression coefficients and their standard errors, allowing the magnitude and direction of the individual effects to be examined. The negative coefficients for methanol concentration and its quadratic term, together with the positive coefficients for extraction time and the methanol concentration × extraction time interaction, indicate a nonlinear response whose dependence on extraction time varies with methanol concentration.

Table 5. ANOVA for the full quadratic RSM describing DPPH radical-scavenging capacity of extracts of original ground coffee [1].

Source	DF	Adj SS	Adj MS	*F*-Value	*p*-Value
Model	9	57,345,197	6,371,689	27.93	<0.001
Linear	3	32,642,001	10,880,667	47.70	<0.001
X_1	1	23,833,882	23,833,882	104.48	<0.001
X_2	1	8,713,739	8,713,739	38.20	<0.001
X_3	1	94,379	94,379	0.41	0.535
Square	3	16,664,751	5,554,917	24.35	<0.001
${X_1}^2$	1	3,247,429	3,247,429	14.24	0.004
${X_2}^2$	1	1,766,931	1,766,931	7.75	0.019
${X_3}^2$	1	98,351	98,351	0.43	0.526
2-Way Interaction	3	8,038,445	2,679,482	11.75	0.001
X_1X_2	1	7,427,724	7,427,724	32.56	<0.001
X_1X_3	1	297,109	297,109	1.30	0.280
X_2X_3	1	313,612	313,612	1.37	0.268
Error	10	2,281,192	228,119	—	—
Lack-of-Fit	5	1,747,382	349,476	3.27	0.110
Pure Error	5	533,810	106,762	—	—
Total	19	59,626,389	—	—	—

[1] X_1, methanol concentration; X_2, extraction time; X_3, extraction temperature; DF, degrees of freedom; Adj SS, adjusted sum of squares; Adj MS, adjusted mean square; *F*-Value, *F*-statistic; *p*-Value, probability value. A *p*-value < 0.05 was considered statistically significant.

Table 6. Estimated coefficients and statistical significance of the full quadratic RSM model for DPPH radical-scavenging capacity of extracts of original ground coffee [1].

Source	Coefficient	SE Coefficient	*T*-Value	*p*-Value
Constant	16,040	164	97.69	<0.001
X_1	−1544	151	−10.22	<0.001
X_2	933	151	6.18	<0.001
X_3	97	151	0.64	0.535
${X_1}^2$	−1087	288	−3.77	0.004
${X_2}^2$	−802	288	−2.78	0.019
${X_3}^2$	−189	288	−0.66	0.526
X_1X_2	964	169	5.71	<0.001
X_1X_3	193	169	1.14	0.280
X_2X_3	198	169	1.17	0.268

[1] X_1, methanol concentration; X_2, extraction time; X_3, extraction temperature; SE, standard error. Coefficients are for the coded variables. A *p*-value < 0.05 was considered statistically significant.

Model adequacy was further assessed using the residual diagnostic plots presented in Figure 2. In the normal probability plot (Figure 2a), most standardized residuals followed the reference line reasonably well, with some deviation at the tails. The standardized

residuals versus fitted values plot (Figure 2b) showed observations distributed on both sides of zero, including one relatively low residual and somewhat greater dispersion at higher fitted values, but no clear systematic pattern. The histogram of standardized residuals (Figure 2c) showed a concentration of observations around zero, with some asymmetry. Examination of the residuals versus observation order (Figure 2d) revealed fluctuations around zero throughout the experimental sequence, without an evident sustained trend.

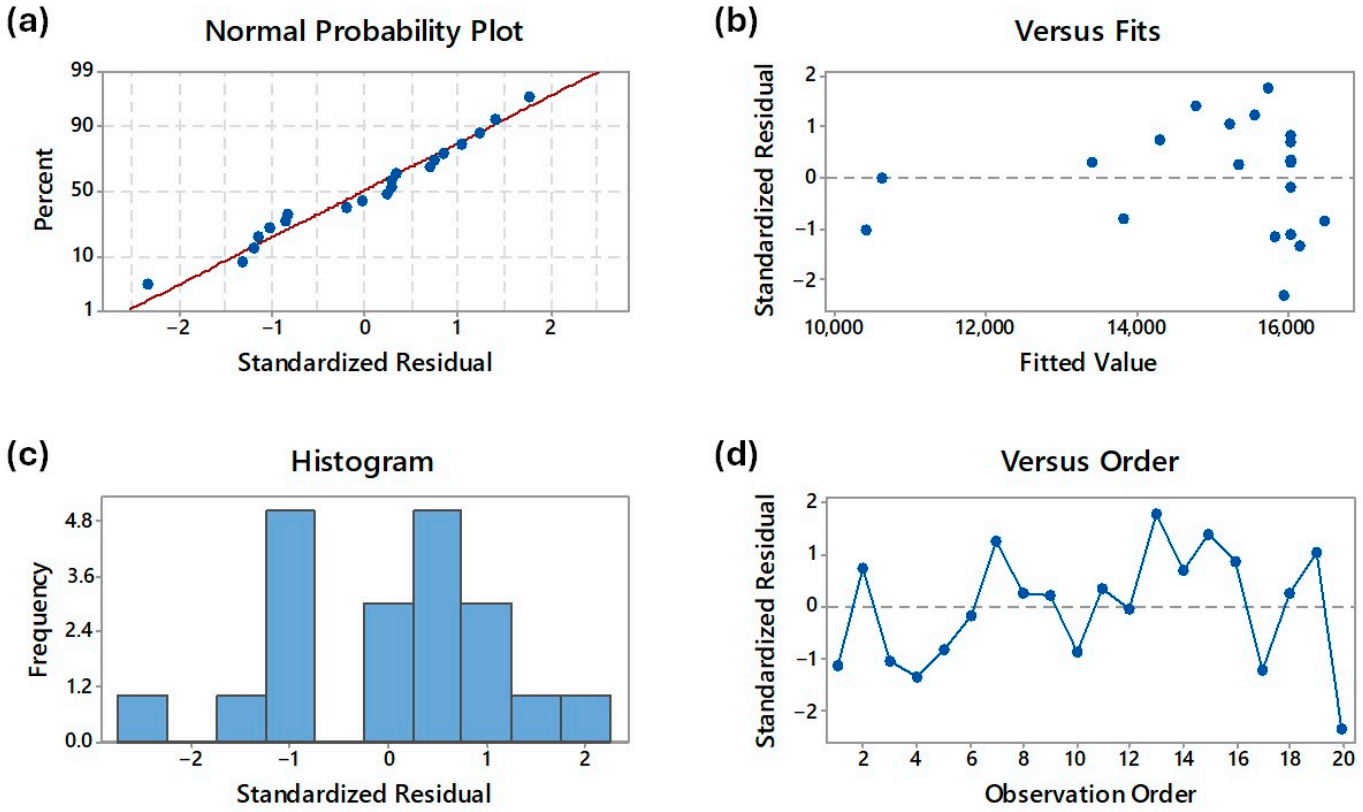


Figure 2. Residual diagnostic plots for the fitted quadratic response surface model of DPPH radical-scavenging capacity: (**a**) normal probability plot, (**b**) standardized residuals versus fitted values, (**c**) histogram of standardized residuals, and (**d**) standardized residuals versus observation order.

The experimental data were fitted to a full second-order polynomial model, expressed in uncoded units as follows (Equation (5)):

$$Y = 14{,}610 - 23.1X_1 + 34.5X_2 + 53.0X_3 - 0.435{X_1}^2 - 0.501{X_2}^2 - 0.84{X_3}^2 + 0.4818X_1X_2 + 0.257X_1X_3 + 0.330X_2X_3 \quad (5)$$

where Y is the predicted DPPH radical-scavenging capacity (mg/kg), X_1 is methanol concentration (% v/v), X_2 is extraction time (min), and X_3 is extraction temperature (°C).

The response surface and contour plots for methanol concentration and extraction time, with temperature fixed at 45 °C, are presented in Figure 3. These graphical representations focus on the methanol concentration × extraction time interaction, as it was statistically significant, whereas interactions involving temperature were not significant. The three-dimensional surface plot (Figure 3a) showed a curved profile, with higher DPPH radical-scavenging capacity concentrated at methanol concentrations below approximately 50% (v/v) and intermediate-to-long extraction times. At 100% (v/v) methanol, the corresponding values remained comparatively modest, particularly at short extraction times. This curvature reflects the nonlinear effects of methanol concentration and extraction time identified by the significant quadratic terms. The contour plot (Figure 3b)

further illustrated the interaction between the two variables, with a broad region exceeding 16,000 mg/kg at methanol concentrations below approximately 50% (v/v) and extraction times above approximately 40 min. The curved contour pattern is consistent with the significant methanol concentration × extraction time interaction identified by the ANOVA.

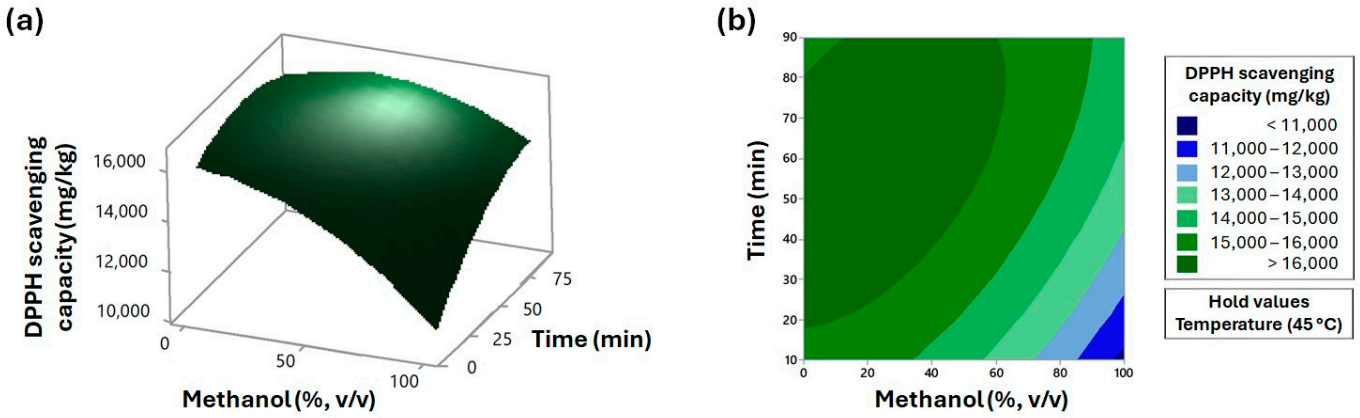


Figure 3. Response surface (**a**) and contour (**b**) plots showing the combined effects of methanol concentration and extraction time on DPPH radical-scavenging capacity at 45 °C.

Numerical optimization was performed using the full quadratic model to maximize DPPH radical-scavenging capacity within the investigated ranges of methanol concentration, extraction time, and temperature. The model identified 22% (v/v) methanol, an extraction time of 61 min, and 47 °C as the numerical optimum, with a predicted response of 16,614 mg/kg. However, the linear and quadratic terms for temperature and its interactions with the other variables were not statistically significant. Thus, the estimated value of 47 °C should be regarded as part of the numerical solution rather than as evidence of a statistically established optimum. Experimental validation under these conditions yielded a DPPH radical-scavenging capacity of 16,039 mg/kg ± 432 mg/kg, within the model's 95% prediction interval of 15,472–17,752 mg/kg. This agreement supports the model's predictive performance at the tested condition.

The preliminary solvent screening showed that water and 50% (v/v) aqueous methanol yielded the highest numerical DPPH radical-scavenging capacities, with no statistically significant difference between them. These findings informed the selection of water and methanol as the solvent components for the subsequent RSM study, which investigated their different proportions alongside extraction time and temperature. The numerical optimum identified by the RSM model (22% v/v methanol, 61 min, and 47 °C) yielded a predicted DPPH radical-scavenging capacity of 16,614 mg/kg. Experimental validation under these conditions resulted in 16,039 mg/kg ± 432 mg/kg, supporting the predictive performance of the model within the investigated experimental domain.

3.2. DPPH Radical-Scavenging Capacity During Sequential Brewing

The DPPH radical-scavenging capacity of the two coffee blends was evaluated during three consecutive brewing cycles to examine changes in the radical-scavenging constituents of the coffee material and the resulting beverages. Results are expressed as gallic acid equivalents, with values reported as mg/kg for ground coffee powder and spent coffee grounds (Table 7), and as mg/L for coffee beverages (Table 8). In Table 7, "original ground coffee" refers to the material before brewing, whereas "spent coffee grounds after Cycle 1" and "spent coffee grounds after Cycle 2" refer to the solid residues recovered after the first and second brewing cycles, respectively. Table 8 reports the beverages obtained during Cycles 1, 2, and 3.

Table 7. DPPH radical-scavenging capacity of original ground coffee and spent coffee grounds recovered after successive brewing cycles [1].

Material	Blend (*w*/*w*)	DPPH Radical-Scavenging Capacity (mg/kg Dry Weight)
Original ground coffee	70% Arabica/30% Robusta	12,444.9 ± 163.8
Original ground coffee	80% Arabica/20% Robusta	16,354.0 ± 214.7
Spent coffee grounds after Cycle 1	70% Arabica/30% Robusta	1598.8 ± 20.1
Spent coffee grounds after Cycle 1	80% Arabica/20% Robusta	1671.1 ± 25.4
Spent coffee grounds after Cycle 2	70% Arabica/30% Robusta	423.3 ± 26.8
Spent coffee grounds after Cycle 2	80% Arabica/20% Robusta	1511.9 ± 60.4

[1] Values are presented as mean ± standard deviation of three independent analytical replicates ($n = 3$).

Table 8. DPPH radical-scavenging capacity of Greek coffee beverages obtained during three successive brewing cycles [1].

Brewing Cycle	Blend (*w*/*w*)	DPPH Radical-Scavenging Capacity (mg/L)
Cycle 1	70% Arabica/30% Robusta	316.4 ± 2.0
Cycle 1	80% Arabica/20% Robusta	308.8 ± 1.6
Cycle 2	70% Arabica/30% Robusta	269.6 ± 2.5
Cycle 2	80% Arabica/20% Robusta	287.4 ± 1.6
Cycle 3	70% Arabica/30% Robusta	16.3 ± 0.8
Cycle 3	80% Arabica/20% Robusta	17.5 ± 0.9

[1] Values are presented as mean ± standard deviation of three independent brewing preparations ($n = 3$).

The original ground coffee samples exhibited DPPH radical-scavenging capacities of 12,444.9 mg/kg ± 163.8 mg/kg for the 70% Arabica/30% Robusta (*w*/*w*) blend and 16,354.0 mg/kg ± 214.7 mg/kg for the 80% Arabica/20% Robusta (*w*/*w*) blend. The original ground coffee of the 80% Arabica/20% Robusta (*w*/*w*) blend exhibited a higher measured DPPH radical-scavenging capacity than the 70% Arabica/30% Robusta (*w*/*w*) blend, while the differences between blends varied in the spent coffee grounds recovered after successive brewing cycles. In the corresponding first-cycle beverages, the values were 316.4 mg/L ± 2.0 mg/L and 308.8 mg/L ± 1.6 mg/L, respectively.

Following the first brewing, measurable DPPH radical-scavenging capacity remained in the spent coffee grounds. The values for spent coffee grounds after Cycle 1 were 1598.8 mg/kg ± 20.1 mg/kg for the 70% Arabica/30% Robusta (*w*/*w*) blend and 1671.1 mg/kg ± 25.4 mg/kg for the 80% Arabica/20% Robusta (*w*/*w*) blend. After the second brewing, the corresponding values for spent coffee grounds after Cycle 2 were 423.3 mg/kg ± 26.8 and 1511.9 mg/kg ± 60.4 mg/kg, respectively. The beverage fraction followed a different pattern, with similar concentrations during Cycle 2 (269.6 mg/L ± 2.5 mg/L and 287.4 mg/L ± 1.6 mg/L) and closely comparable concentrations in Cycle 3 (16.3 mg/L ± 0.8 mg/L and 17.5 mg/L ± 0.9 mg/L) for the 70% and 80% Arabica (*w*/*w*) materials, respectively.

The concentration-dependent DPPH measurements obtained for the ground coffee and spent coffee grounds are presented in Figure 4. Increasing sample content resulted in progressively greater radical-scavenging responses within the tested concentration ranges. The regression relationships illustrate differences in the concentration–response behavior between the initial ground coffee and the spent coffee grounds used as inputs in the subsequent brewing cycles. Consequently, the percentage values obtained at different sample contents were used to characterize the concentration–response behavior rather than as direct measures for comparing samples tested under different assay conditions.

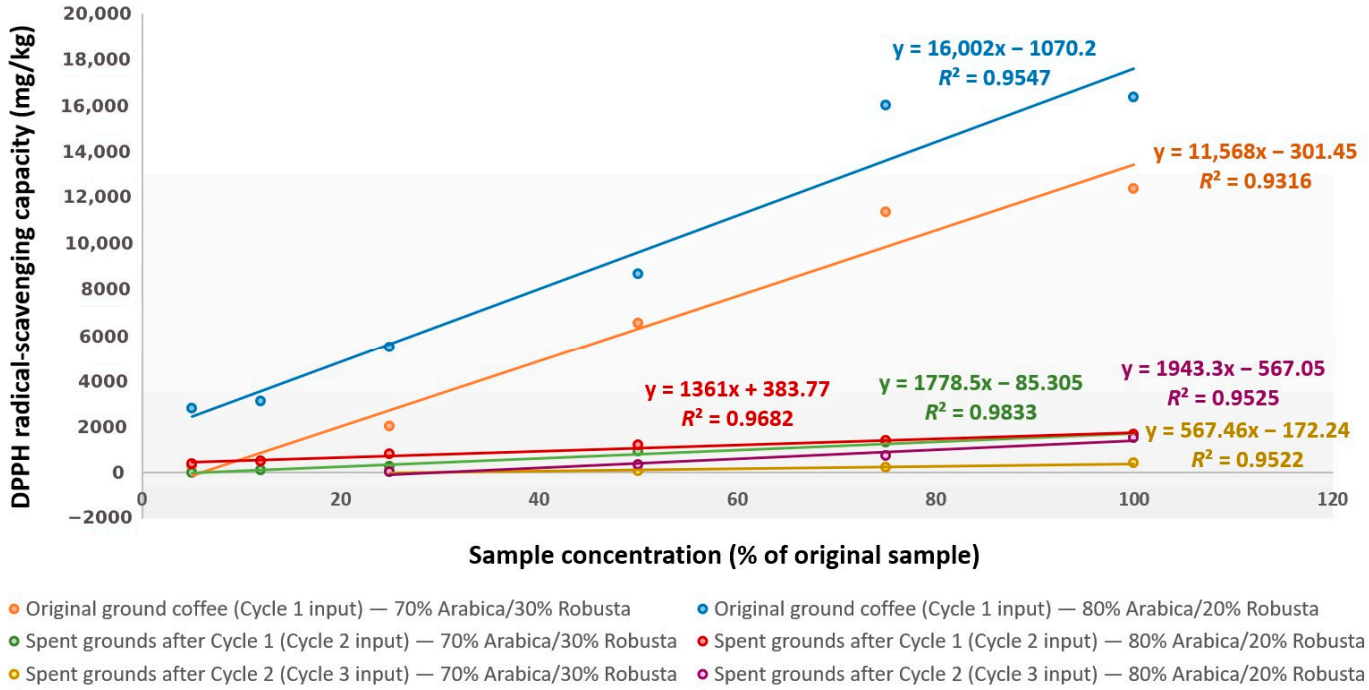


Figure 4. Concentration–response relationships between sample content and DPPH radical-scavenging capacity for the solid coffee materials used as inputs in three successive Greek coffee brewing cycles. The materials comprised original ground coffee used in Cycle 1, spent coffee grounds recovered after Cycle 1 and used in Cycle 2, and spent coffee grounds recovered after Cycle 2 and used in Cycle 3. Both coffee blends (70% Arabica/30% Robusta and 80% Arabica/20% Robusta, w/w) are presented. Linear regression equations and coefficients of determination (R^2) are shown for each sample. For the x-axis, 100% represents the original, undiluted sample.

The corresponding concentration–response relationships for the coffee beverages are presented in Figure 5. The radical-scavenging response increased with increasing sample content, while the magnitude of the response varied between brewing cycles and coffee blends. The Cycle 3 beverages exhibited markedly weaker responses across the tested sample-content range than the corresponding first- and second-cycle beverages. These concentration–response profiles are consistent with the differences in gallic acid-equivalent DPPH radical-scavenging capacity reported in Table 8.

Sequential brewing was associated with substantial changes in DPPH radical-scavenging capacity in both the solid and beverage fractions. In the solid fraction, the measured capacity was lower in the spent coffee grounds than in the original ground coffee, with the magnitude of the decrease differing between the two blends. In the beverage fraction, the measured capacity decreased across the three brewing cycles, with relatively similar values between the blends in Cycles 2 and 3.

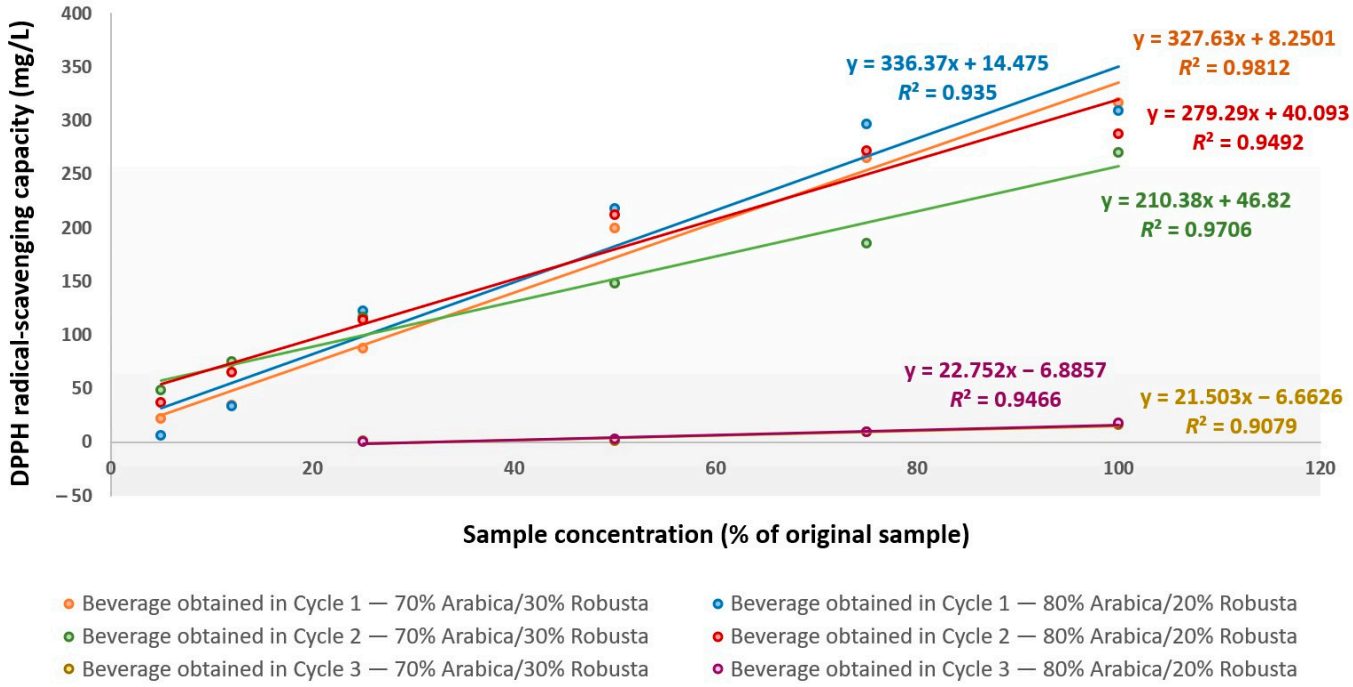


Figure 5. Concentration–response relationships between sample content and DPPH radical-scavenging capacity for the coffee beverages obtained during three successive Greek coffee brewing cycles. Beverages are identified according to the brewing cycle in which they were produced (Cycles 1, 2 and 3) and the corresponding coffee blend (70% Arabica/30% Robusta and 80% Arabica/20% Robusta, w/w). Linear regression equations and coefficients of determination (R^2) are shown for each sample. For the x-axis, 100% represents the original, undiluted sample.

3.3. Total Phenolic Content During Sequential Brewing

The total phenolic content decreased across successive brewing cycles in both the solid and beverage fractions (Tables 9 and 10). The spent coffee grounds showed lower total phenolic content than the original ground coffee, with a further decrease in the residues recovered after the second brewing. The beverage fraction also showed a progressive decrease in total phenolic content across the three brewing cycles. In Table 9, "spent coffee grounds after Cycle 1" and "spent coffee grounds after Cycle 2" refer to the solid residues recovered after the first and second brewing cycles, respectively, whereas Table 10 reports the beverages obtained during Cycles 1, 2, and 3.

Table 9. Total phenolic content of original ground coffee and spent coffee grounds recovered after successive brewing cycles [1].

Material	Blend (w/w)	Total Phenolic Content (mg/kg, Dry Weight)
Original ground coffee	70% Arabica/30% Robusta	13,724.0 ± 330.5
Original ground coffee	80% Arabica/20% Robusta	13,604.0 ± 361.7
Spent coffee grounds after Cycle 1	70% Arabica/30% Robusta	1834.4 ± 42.1
Spent coffee grounds after Cycle 1	80% Arabica/20% Robusta	3522.4 ± 75.0
Spent coffee grounds after Cycle 2	70% Arabica/30% Robusta	538.4 ± 33.0
Spent coffee grounds after Cycle 2	80% Arabica/20% Robusta	1418.4 ± 63.2

[1] Values are presented as mean ± standard deviation of three independent analytical replicates ($n = 3$).

Table 10. Total phenolic content of Greek coffee beverages obtained during three successive brewing cycles [1].

Brewing Cycle	Blend (w/w)	Total Phenolic Content (mg/L)
Cycle 1	70% Arabica/30% Robusta	15,532.4 ± 130.0
Cycle 1	80% Arabica/20% Robusta	13,409.1 ± 143.2
Cycle 2	70% Arabica/30% Robusta	940.5 ± 18.3
Cycle 2	80% Arabica/20% Robusta	933.6 ± 21.0
Cycle 3	70% Arabica/30% Robusta	253.2 ± 10.4
Cycle 3	80% Arabica/20% Robusta	344.2 ± 18.2

[1] Values are presented as mean ± standard deviation of three independent brewing preparations ($n = 3$).

The original ground coffee samples showed similar measured total phenolic contents. The measured values in the spent coffee grounds were lower than those in the original ground coffee, with the 80% Arabica/20% Robusta (w/w) blend showing higher values than the 70% Arabica/30% Robusta (w/w) blend after both the first and second brewing cycles. The values observed in the spent grounds after the second brewing of the 70% Arabica/30% Robusta blend were similar in magnitude to those observed after the first brewing of the 80% Arabica/20% Robusta blend. In any case, the findings are descriptive and do not imply statistically significant differences between the blends.

The beverage fraction showed a marked decrease in measured total phenolic content across successive brewing cycles. The two blends had similar numerical values in Cycle 2, whereas the 80% Arabica/20% Robusta (w/w) blend had a higher numerical value in Cycle 3. These observations describe the measured patterns and should not be interpreted as evidence of statistically significant differences between blends or brewing cycles.

Pearson's correlation analysis was performed to assess the relationship between total phenolic content, determined using the Folin–Ciocalteu assay, and DPPH radical-scavenging capacity in the solid and beverage fractions separately. A strong positive correlation was found for the solid samples ($r = 0.964$, $p < 0.001$), compared with a moderate positive correlation for the coffee beverages ($r = 0.615$, $p = 0.007$). Despite these overall associations, the two measurements differed considerably in some individual samples. For example, in the 80% Arabica/20% Robusta (w/w) blend, the spent coffee grounds recovered after the first brewing had a total phenolic content of 3522.4 mg/kg and a DPPH radical-scavenging capacity of 1671.1 mg/kg. These findings indicate that the two assays did not show identical patterns across all samples.

3.4. Residual Moisture Content of Spent Coffee Grounds

As shown in Table 11, residual moisture content increased from the original ground coffee to the spent coffee grounds recovered after successive brewing cycles for both coffee blends, despite the same drying treatment (45 °C for 12 h). The 80% Arabica/20% Robusta blend consistently exhibited slightly higher residual moisture than the 70% Arabica/30% Robusta blend, although the differences between blends were small compared with the increase across the sample sequence. This pattern indicates that the specified drying treatment did not achieve a uniform residual moisture level in the grounds recovered at different stages of sequential brewing. Changes in the physical characteristics of the grounds and their water-retention properties may contribute to this trend, although these mechanisms were not directly investigated.

Table 11. Residual moisture content of original ground coffee and spent coffee grounds recovered after successive Greek coffee brewing cycles [1].

Material	Blend (w/w)	Moisture (%, w/w)
Original ground coffee	70% Arabica/30% Robusta	2.21 ± 0.08
Original ground coffee	80% Arabica/20% Robusta	2.44 ± 0.12
Spent coffee grounds after Cycle 1	70% Arabica/30% Robusta	5.43 ± 0.17
Spent coffee grounds after Cycle 1	80% Arabica/20% Robusta	5.77 ± 0.15
Spent coffee grounds after Cycle 2	70% Arabica/30% Robusta	7.79 ± 0.20
Spent coffee grounds after Cycle 2	80% Arabica/20% Robusta	8.69 ± 0.29

[1] Values are presented as mean ± standard deviation of three independent analytical replicates ($n = 3$).

3.5. pH During Sequential Brewing

Table 12 reports the pH of the coffee beverages obtained during each brewing cycle. The two blends showed similar pH values within each cycle, while the measured values increased progressively from Cycle 1 to Cycle 3. These observations describe the pH patterns associated with sequential brewing; inferential comparisons between blends and cycles were not performed.

Table 12. pH of Greek coffee beverages obtained during three successive brewing cycles [1].

Brewing Cycle	Blend (w/w)	pH Value
Cycle 1	70% Arabica/30% Robusta	4.92 ± 0.07
Cycle 1	80% Arabica/20% Robusta	5.15 ± 0.12
Cycle 2	70% Arabica/30% Robusta	5.90 ± 0.16
Cycle 2	80% Arabica/20% Robusta	6.01 ± 0.04
Cycle 3	70% Arabica/30% Robusta	6.35 ± 0.16
Cycle 3	80% Arabica/20% Robusta	6.40 ± 0.05

[1] Values are presented as mean ± standard deviation of three independent brewing preparations ($n = 3$).

3.6. Turbidity and Sedimentation During Sequential Brewing

Optical density (OD) was used as an indicator of beverage turbidity, while the sedimentation rate (R_s) was used to characterize the rate at which suspended material settled during the measurement period. Differences in initial optical density (OD_0) and optical density after sedimentation (OD_t) were observed among the six coffee beverages (Table 13).

Table 13. Optical density and sedimentation rate of Greek coffee beverages obtained during three successive brewing cycles [1].

Brewing Cycle	Blend (w/w)	OD_0	OD_t	R_s (min^{-1})
Cycle 1	70% Arabica/30% Robusta	2.521 ± 0.010	0.561 ± 0.011	0.098 ± 0.000
Cycle 1	80% Arabica/20% Robusta	3.040 ± 0.019	1.298 ± 0.009	0.087 ± 0.001
Cycle 2	70% Arabica/30% Robusta	2.678 ± 0.013	0.688 ± 0.008	0.100 ± 0.001
Cycle 2	80% Arabica/20% Robusta	2.398 ± 0.008	0.940 ± 0.008	0.073 ± 0.001
Cycle 3	70% Arabica/30% Robusta	2.302 ± 0.007	0.159 ± 0.010	0.107 ± 0.000
Cycle 3	80% Arabica/20% Robusta	1.991 ± 0.009	0.312 ± 0.010	0.084 ± 0.000

[1] Values are presented as mean ± standard deviation of three independent brewing preparations ($n = 3$).

The highest OD_0 value was recorded for the 80% Arabica/20% Robusta (w/w) blend in Cycle 1 (3.040), whereas the lowest was observed for the same blend in Cycle 3 (1.991). For the 70% Arabica/30% Robusta (w/w) blend, OD_0 increased from Cycle 1 to Cycle 2 before decreasing in Cycle 3. OD_t was lower in Cycle 3 than in the preceding cycles for both blends.

Sedimentation rates varied numerically among the six beverage samples. The highest measured rate occurred for the 70% Arabica/30% Robusta (*w*/*w*) blend in Cycle 3, whereas the lowest occurred for the 80% Arabica/20% Robusta (*w*/*w*) blend in Cycle 2. These observations indicate variation in sedimentation behavior across the samples, but no inferential comparisons between blends or brewing cycles were performed.

The sedimentation profiles of the two coffee blends are presented in Figure 6. For the 70% Arabica/30% Robusta (*w*/*w*) beverage, the profiles illustrate changes in turbidity during sedimentation across the three brewing cycles (Figure 6a). The corresponding profiles for the 80% Arabica/20% Robusta (*w*/*w*) beverage are shown in Figure 6b, illustrating differences in turbidity reduction and sedimentation behavior among the cycles. These profiles are consistent with the differences in OD_0, OD_t, and R_s reported in Table 13.

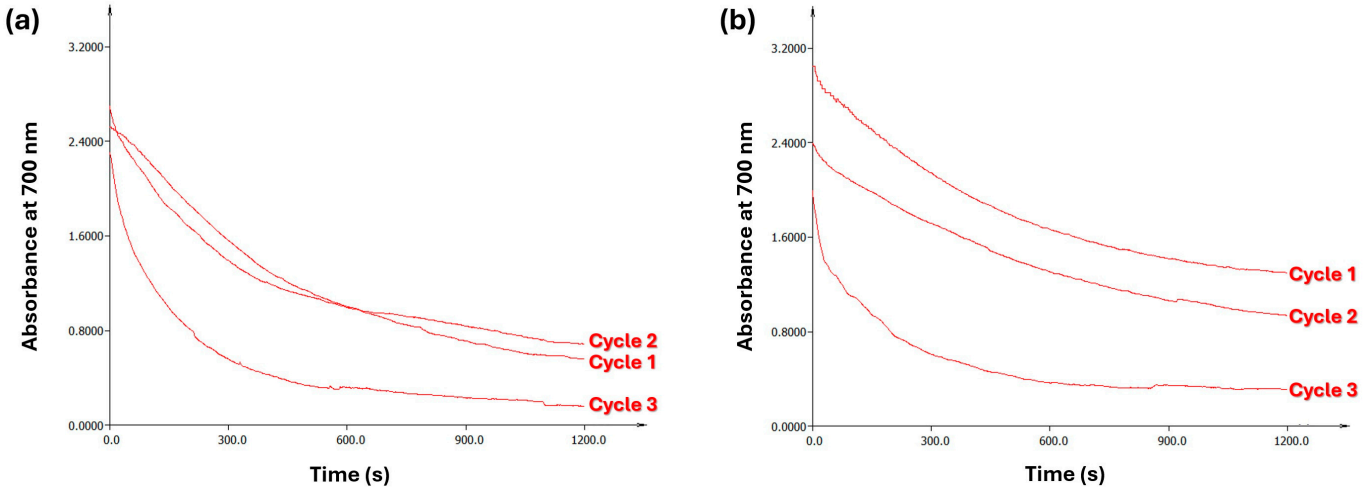


Figure 6. Optical density profiles during sedimentation of coffee beverages across three brewing cycles, representing (**a**) the 70% Arabica/30% Robusta (*w*/*w*) blend and (**b**) the 80% Arabica/20% Robusta (*w*/*w*) blend.

3.7. Microscopic Characterization During Sequential Brewing

Microscopic examination revealed visible differences in particle appearance across brewing cycles and between the two coffee blends. A qualitative assessment was performed, focusing on particle shape, apparent size, color, and the presence of closely associated particles or clusters.

The solid samples examined microscopically correspond to the materials used as inputs in the three successive brewing cycles. Accordingly, the original ground coffee represents the input to Cycle 1, the spent coffee grounds recovered after Cycle 1 represent the input to Cycle 2, and the spent coffee grounds recovered after Cycle 2 represent the input to Cycle 3. The final spent coffee grounds recovered after Cycle 3 were not included in the microscopic analysis.

The 10× micrographs (Figure 7) show numerous small particles dispersed throughout the fields, interspersed with larger dark particles and irregularly shaped clusters. For the original ground coffee blends used in Cycle 1, the 70% Arabica/30% Robusta (*w*/*w*) sample contains a conspicuous elongated structure near the left side of the field, whereas the 80% Arabica/20% Robusta (*w*/*w*) sample contains a prominent rounded dark particle near the upper central region. The spent coffee grounds used as input to Cycle 2 contain numerous fine particles together with several larger, irregular structures, particularly toward the lower portions of the images. The spent coffee grounds used as input to Cycle 3 show that larger dark structures remain visible among the finer material, with the 80% Arabica/20% Robusta (*w*/*w*) sample showing several closely associated particles forming irregular arrangements.

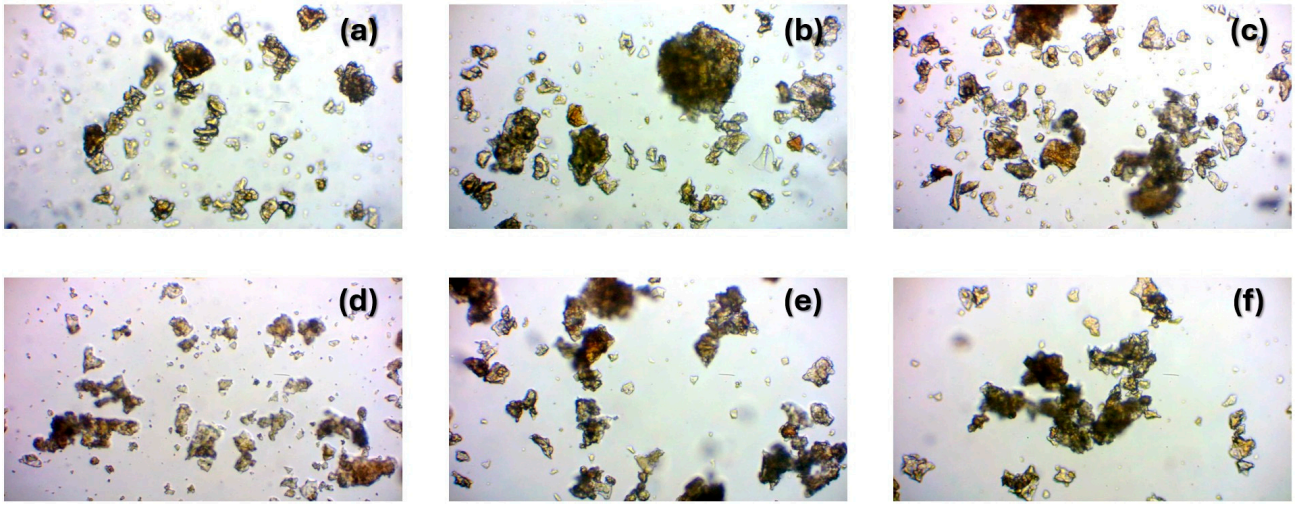


Figure 7. Representative light microscopy images of the solid coffee materials used as inputs in three successive Greek coffee brewing cycles, at 10× magnification. Panels (**a**,**b**) correspond to the original ground coffee used in Cycle 1 for the 70% Arabica/30% Robusta and 80% Arabica/20% Robusta blends, respectively; panels (**c**,**d**) correspond to the spent coffee grounds recovered after Cycle 1 and used in Cycle 2; and panels (**e**,**f**) correspond to the spent coffee grounds recovered after Cycle 2 and used in Cycle 3.

At 40× magnification (Figure 8), the individual particles show more clearly visible morphological features. The original ground coffee used in Cycle 1 contains angular to irregular particles with brown-yellow regions and more translucent peripheral areas. The spent coffee grounds used as input to Cycle 2 show broad angular forms and larger irregular structures, with variations in color and visible internal features. For the spent coffee grounds used as input to Cycle 3, the 70% Arabica/30% Robusta (w/w) sample contains a large, irregular, elongated structure with a mottled appearance, whereas the 80% Arabica/20% Robusta (w/w) sample shows a cluster of closely associated particles with darker brown regions. The 40× images therefore show differences in apparent size, color, and the proximity of neighboring particles.

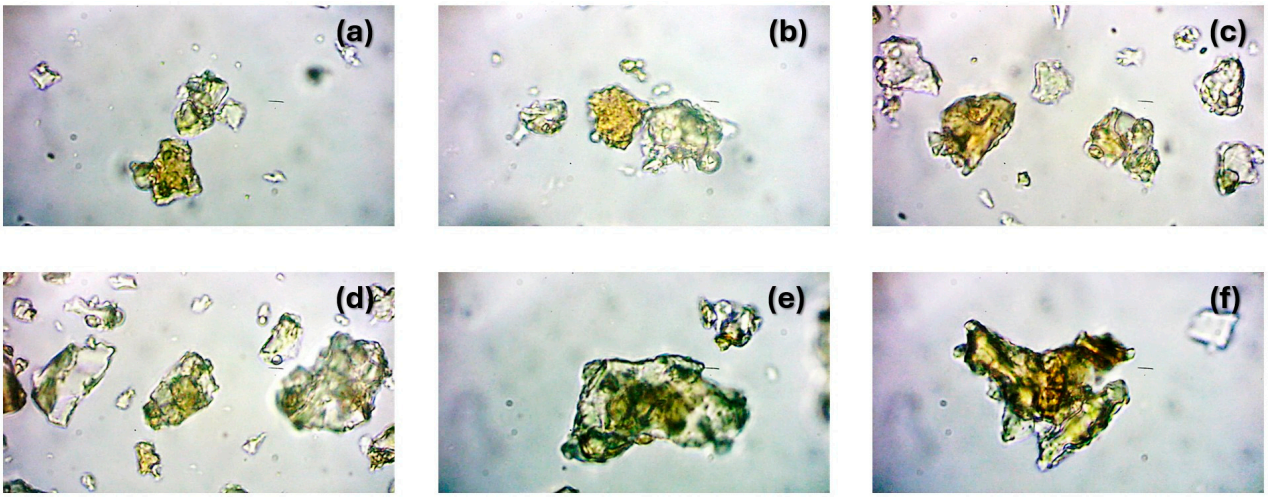


Figure 8. Representative light microscopy images of the solid coffee materials used as inputs in three successive Greek coffee brewing cycles, at 40× magnification. Panels (**a**,**b**) correspond to the original ground coffee used in Cycle 1 for the 70% Arabica/30% Robusta and 80% Arabica/20% Robusta blends, respectively; panels (**c**,**d**) correspond to the spent coffee grounds recovered after Cycle 1 and used in Cycle 2; and panels (**e**,**f**) correspond to the spent coffee grounds recovered after Cycle 2 and used in Cycle 3.

3.8. Consumer Perceptions and Acceptance of Coffee-Ground Valorization

A total of 82 respondents participated in the consumer survey. The sample comprised 63.4% women and 36.6% men. Participants aged more than 55 years represented 34.1% of the sample, followed by those aged 46–55 years (32.9%), 36–45 years (13.4%), 18–25 years (12.2%), and 26–35 years (7.3%). Most respondents were employed (86.6%) and lived in urban areas (79.3%). Regarding education, 31.7% had completed upper secondary education, 29.3% held an undergraduate degree, 25.6% had postgraduate or doctoral education, and 13.4% had vocational training.

Greek coffee consumption was reported as daily by 37.8% of respondents, while 35.4% consumed it rarely, 18.3% consumed it 1–2 times per week, and 8.5% consumed it 3–5 times per week. Most participants consumed 0–1 cups per day (69.5%), followed by two cups (23.2%) and three or more cups (7.3%). Traditional *briki* preparation was predominant (74.4%), whereas 23.2% used an electric *briki*. Knowledge of the coffee blend was relatively limited, with 59.8% reporting that they did not know which type of blend they consumed; 28.0% identified their coffee as primarily Arabica, 9.8% as an Arabica–Robusta blend, and 2.4% as mainly Robusta.

Awareness of the potential bioactive properties of Greek coffee was relatively high. As shown in Figure 9, 75.6% of respondents had heard that Greek coffee contains natural antioxidant compounds, while 24.4% had not. Similarly, 76.8% believed that spent coffee grounds may contain natural compounds that could be utilized in other applications, such as cosmetics or circular-economy products. Despite this perception, 74.4% reported that they normally discard their spent coffee grounds, whereas 25.6% reported another use.

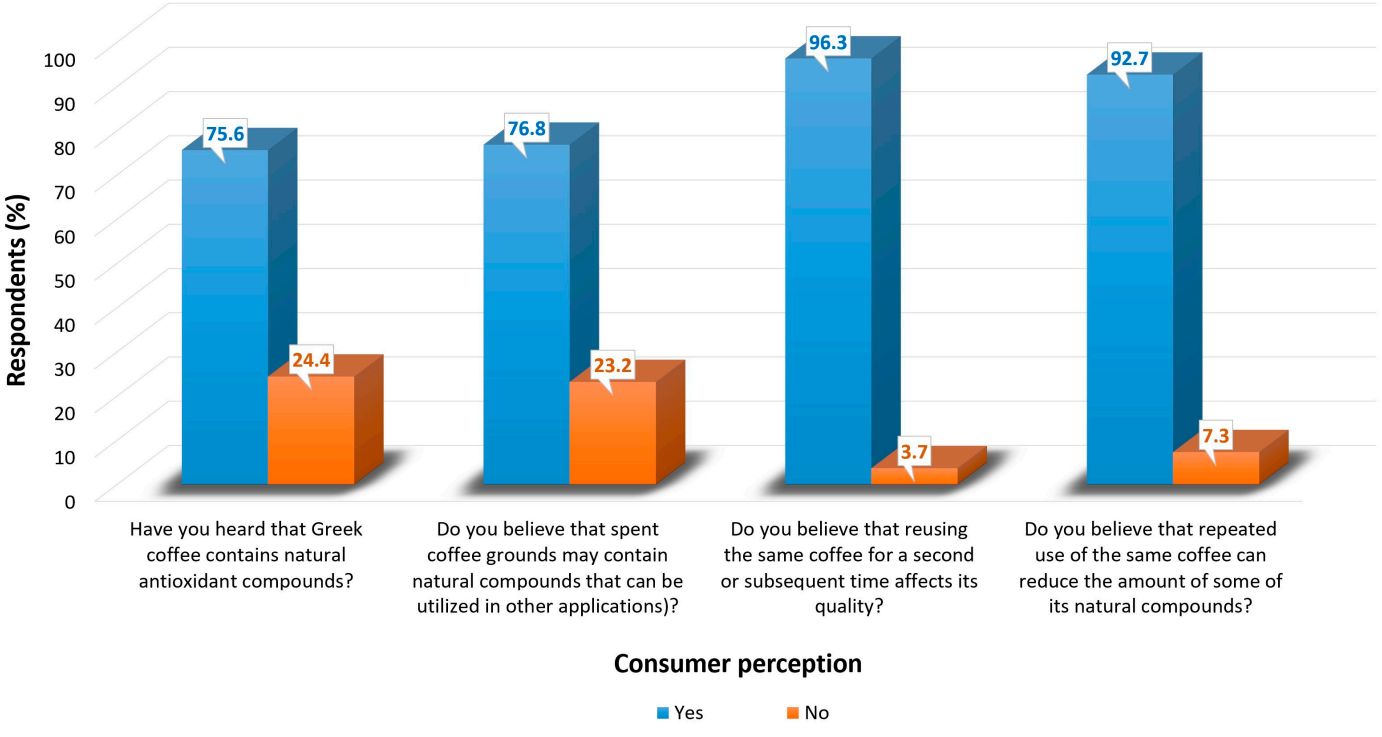


Figure 9. Consumer awareness and perceptions concerning Greek coffee and repeated use of coffee grounds. Bars represent the percentage of respondents selecting each response for awareness of natural antioxidant compounds, perceived potential of spent coffee grounds for other applications, perceived effect of repeated use on coffee quality, and perceived reduction in natural compounds following repeated use.

The large majority of respondents (96.3%) believed that a second or subsequent use reduces coffee quality, while only 3.7% considered that quality remains unchanged. Likewise,

92.7% believed that repeated use can reduce the amount of natural compounds, compared with 7.3% who did not share this view (Figure 9). In response to a separate questionnaire item on perceived health benefits, 78.0% of respondents believed that consumption of Greek coffee may be associated with health benefits.

The four five-point ordinal measures provided further information on consumer attitudes (Table 14). The importance assigned to antioxidant activity had a median score of 4 (IQR = 2), with 65.8% of respondents selecting scores of 4 or 5. Trust in scientific studies concerning the potential benefits of coffee also showed a median of 4 (IQR = 1), with 59.7% selecting 4 or 5. The importance assigned to sustainable valorization of food residues had a median of 4 (IQR = 2), with 62.1% selecting 4 or 5. In comparison, purchase intention for products derived from coffee-residue valorization was more moderate, with a median of 3 (IQR = 1); 43.9% of respondents selected scores of 4 or 5.

Table 14. Descriptive statistics of consumer attitude measures [1].

Consumer Attitude Measure	Median	IQR	Min–Max
Importance of antioxidant activity	4	2	1–5
Trust in scientific studies regarding potential coffee benefits	4	1	2–5
Purchase intention for products derived from coffee-residue valorization	3	1	1–5
Importance of sustainable valorization of food residues	4	2	1–5

[1] Values are based on responses from 82 participants. IQR, interquartile range; Min–Max, minimum–maximum score. All measures were assessed on a five-point ordinal scale (1–5).

The full response distributions for these four ordinal measures are presented in Figure 10. The distributions show that antioxidant activity and sustainable residue valorization were generally assigned high importance, whereas responses concerning purchase intention were more concentrated around the intermediate category.

Despite the relatively high recognition of the potential value of spent coffee grounds, direct acceptance of a coffee prepared using residue-valorization technologies was limited (Figure 11). Only 5 respondents (6.1%) stated that they would consume such a coffee, whereas 34 (41.5%) selected “Maybe” and 43 (52.4%) responded “No”.

Spearman’s correlation analysis identified three statistically significant positive associations among the four ordinal attitude measures (Figure 12). The importance assigned to antioxidant activity was positively correlated with trust in scientific studies regarding potential coffee benefits ($\rho = 0.232$, $p = 0.036$). Trust in scientific studies was also positively associated with purchase intention for products derived from coffee-residue valorization ($\rho = 0.261$, $p = 0.018$). The strongest association was observed between purchase intention and the importance assigned to sustainable valorization of food residues ($\rho = 0.623$, $p < 0.001$). The remaining associations were not statistically significant: antioxidant-activity importance with purchase intention ($\rho = 0.102$, $p = 0.364$), antioxidant-activity importance with sustainable valorization ($\rho = 0.174$, $p = 0.118$), and scientific-study trust with sustainable valorization ($\rho = 0.147$, $p = 0.188$).

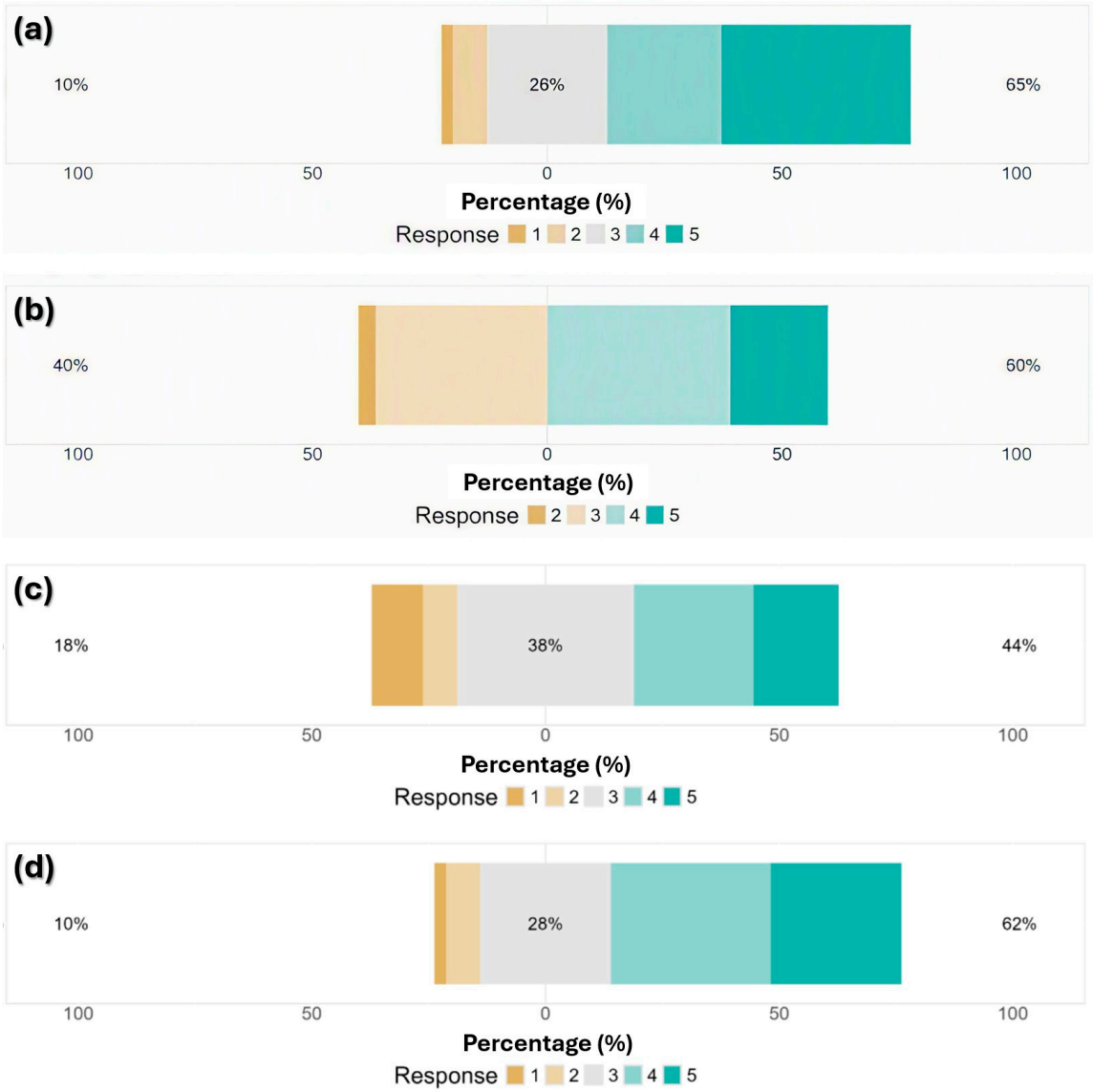


Figure 10. Distribution of responses to the four five-point consumer attitude measures. (**a**) importance of antioxidant activity when selecting a food or beverage; (**b**) trust in scientific studies regarding potential coffee benefits; (**c**) intention to purchase products derived from coffee-residue valorization; and (**d**) importance assigned to sustainable valorization of food residues. Values are percentages of respondents ($n = 82$).

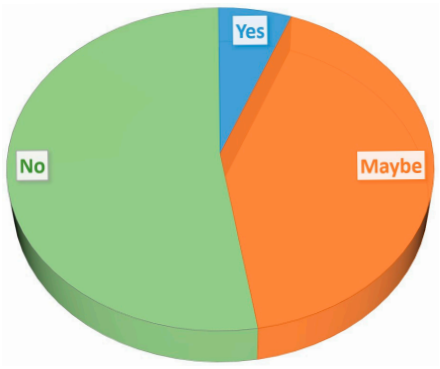


Figure 11. Willingness to consume Greek coffee prepared using coffee-residue valorization technologies. Responses are presented as percentages of respondents ($n = 82$).

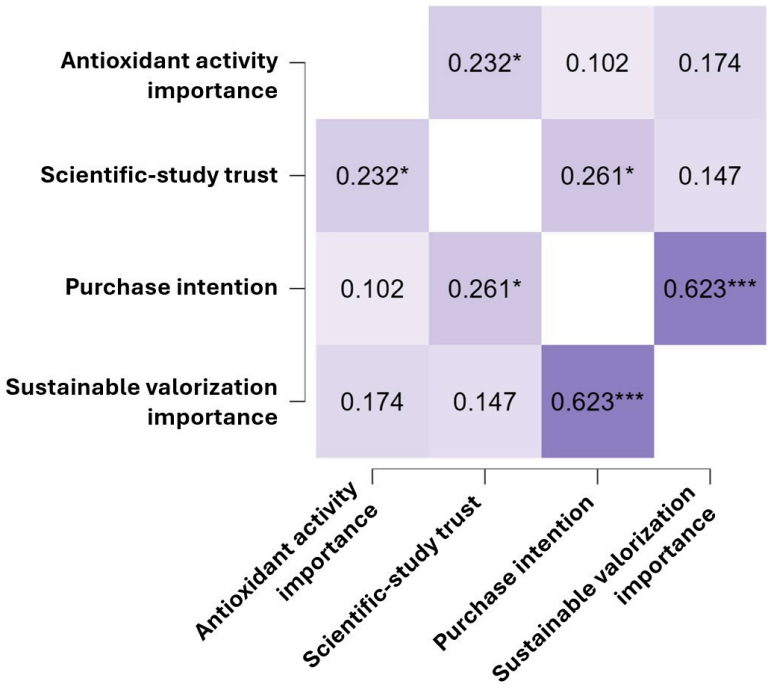


Figure 12. Spearman's correlation heatmap for consumer attitude measures. Values represent Spearman's ρ. Statistical significance is indicated by asterisks: * $p < 0.05$; *** $p < 0.001$.

4. Discussion

The extraction analysis indicates that solvent composition and extraction time were the principal factors governing recovery of radical-scavenging constituents from the coffee residue matrix, whereas the investigated temperature range did not exert a significant effect. This differs from studies in which temperature was a significant determinant of phenolic recovery, highlighting the dependence of extraction-variable effects on the extraction system and response measured [33–35]. Preliminary solvent screening identified water as yielding the highest numerical DPPH radical-scavenging capacity, followed closely by 50% (v/v) aqueous methanol, with no statistically significant difference between these two solvent systems. The RSM optimum at 22% (v/v) methanol further indicates that high antioxidant recovery was achieved with an aqueous mixture rather than the pure organic solvent. This difference reflects the distinct purposes of the two stages: preliminary screening compared solvents under fixed conditions, whereas RSM investigated methanol concentration, extraction time, and temperature simultaneously. Moreover, the significant interaction between methanol concentration and extraction time indicates that the effect of methanol concentration depended on extraction duration, helping to explain why the RSM optimum differed from the numerical ranking observed during preliminary screening. Although numerical optimization returned 47 °C as the temperature setting, the nonsignificant temperature-related model terms do not support interpreting this value as a distinct optimum. The observed behavior can be explained by the role of solvent polarity and solute–solvent interactions in determining the solubility of compounds with different physicochemical characteristics [36,37].

Methanol is widely used for the analytical extraction of phenolic compounds and has generally shown higher extraction efficiency for lower-molecular-weight polyphenols than other commonly used solvents [38]. In the present study, methanol was selected as an analytical solvent to assess the radical-scavenging potential of spent coffee grounds generated by brewing, filtration, and drying. It was not proposed as a solvent for producing a food ingredient intended for consumption. Under EU Directive 2009/32/EC, methanol is

listed as an extraction solvent subject to a maximum residue limit of 10 mg/kg in the extracted foodstuff or food ingredient, whereas ethanol is listed for use in accordance with good manufacturing practice [39]. Accordingly, the use of methanol for analytical characterization should be distinguished from the selection of solvents for food processing. For potential food-related applications, ethanol–water mixtures may offer a more suitable alternative, although their extraction performance would require independent evaluation under the relevant conditions. Previous studies on spent coffee grounds support investigating such mixtures, reporting favorable phenolic recovery with 60% (v/v) ethanol in water compared with pure ethanol or water [35], while 68% (v/v) ethanol was identified as optimal under microwave-assisted extraction [40].

Ultrasound-assisted extraction has been investigated as a means of recovering bioactive compounds from spent coffee grounds [17]. In the present study, however, it was used exclusively as an analytical sample-preparation technique to characterize the residual material and did not constitute part of the investigated valorization strategy. Within this analytical system, the absence of a significant temperature effect indicates that increasing the temperature did not improve the measured radical-scavenging response within the investigated range. This finding is relevant to the selection of analytical extraction conditions, although it should not be interpreted as evidence of an environmental advantage, since the energy requirements of the complete procedure, including drying and solvent recovery, were not determined.

The progressive decreases in total phenolic content and radical-scavenging capacity across brewing cycles may reflect the preferential removal of water-soluble bioactive constituents during the initial brewing, followed by a smaller remaining fraction in subsequent cycles [15]. Nonetheless, measurable phenolic and radical-scavenging capacity persisted after repeated brewing, indicating that assay-detectable constituents remained in the residual grounds. The spent coffee grounds recovered after the first brewing retained measurable phenolic content and radical-scavenging capacity, while the grounds recovered after the second brewing showed further depletion. The two commercial blends also showed distinct residual profiles, suggesting that the initial coffee matrix influences constituent retention. Previous comparative studies have recorded differences in phenolic composition and antioxidant characteristics between Arabica- and Robusta-derived materials, with Robusta generally showing higher levels of several phenolic constituents and antioxidant responses, although the magnitude of these differences varies with coffee origin and processing conditions [41–43]. Yet, the two samples also differed in roast level, making it difficult to determine whether the observed differences arose from the Arabica/Robusta ratio or from roasting.

Although total phenolic content and DPPH radical-scavenging capacity were positively correlated, their relationship was not uniform across all samples. Differences between these measurements may reflect the distinct analytical responses of the two assays, as the Folin–Ciocalteu reagent can react with certain non-phenolic reducing compounds [44]. Variations in the association between total phenolic content and DPPH radical-scavenging capacity have also been observed in coffee samples subjected to different roasting levels [45]. These findings indicate that total phenolic content alone may not fully reflect the DPPH radical-scavenging capacity of coffee extracts.

Beyond the bioactive content, repeated brewing modified several characteristics of the coffee residue and beverage. The progressive increase in beverage pH may reflect differences in the extraction of acidic compounds during successive brewing cycles, as preparation conditions influence the extraction of organic acids and other compounds contributing to coffee acidity [46]. The heterogeneous particle morphology observed microscopically is compatible with the irregular morphology reported for spent coffee grounds [47]. Recent

evidence from higher-resolution imaging has shown increased porosity, cell-wall disruption, and surface fragmentation following repeated brewing of spent coffee grounds [14,15], which could facilitate continued interaction between the remaining matrix and the brewing medium. However, the present optical microscopy cannot establish such structural mechanisms directly.

Turbidity and sedimentation showed variation across brewing cycles and between the two coffee blends. For the 70% Arabica/30% Robusta (w/w) blend, the sedimentation rates showed relatively similar numerical values in Cycles 1 and 2, followed by a higher value in Cycle 3. For the 80% Arabica/20% Robusta (w/w) blend, the lowest numerical rate was observed in Cycle 2. Turbidity reflects the optical scattering of suspended particles, and its relationship with concentration is affected by particle size and optical properties [48]. Sedimentation is additionally influenced by particle and aggregate characteristics, including particle size, density, aggregation, and settling velocity [49]. The observed differences between turbidity and sedimentation suggest that suspended material concentration alone did not determine settling behavior. This interpretation remains tentative, as particle-size distribution and aggregation were not quantitatively characterized. No inferential statistical comparisons were performed.

From a circular-economy perspective, this study examined the sequential reuse of spent coffee grounds in successive Greek coffee preparations as its sole valorization strategy. Characterization across brewing cycles showed that the residual grounds retained measurable phenolic content and radical-scavenging capacity after reuse. Repeated brewing therefore extends the use of the original coffee material before its final disposal. Other studies have explored broader valorization pathways, including the recovery of value-added products [50] and the development of alternative applications [51,52], which were beyond the scope of the present work. The practical benefits of sequential reuse will depend, however, on whether the resources required for further brewing are justified by the properties and potential use of the resulting beverages. Process scalability and environmental burdens therefore require further evaluation [53]. Such an assessment should also account for energy and material inputs, system boundaries, and sensitivity to operating conditions [54].

The consumer findings provide an initial indication of how local consumers in Greece perceive the potential reuse of spent coffee grounds in a Greek coffee product. The association between sustainability orientation and purchase intention suggests that environmental considerations may contribute to consumer acceptance, while the substantial proportion of respondents selecting "Maybe" suggests openness or uncertainty rather than clear rejection. However, sustainability alone may not determine willingness to engage with foods derived from residue valorization. This distinction is relevant for coffee-residue applications, where acceptance may depend on the sensory characteristics and perceived quality of the resulting product, as well as consumers' familiarity with the product and its consumption context [55,56]. The association with trust in science further suggests that confidence in scientific and technological processes may contribute to purchase intention, although its weaker association indicates that it is unlikely to be the sole determinant of consumer responses. Importantly, the present survey assessed a hypothetical application rather than a defined food product. Consequently, the findings should not be interpreted as rejection of coffee-residue valorization itself, because participants could not evaluate a specific product in terms of sensory characteristics, safety assurance, or functional benefits. Consumer acceptance should therefore be examined at the product level, where environmental information can be considered alongside the characteristics that directly shape perceptions of the resulting food.

Several limitations constrain the interpretation and transferability of these findings. The use of two commercial Greek coffee blends and one standardized brewing procedure

limits the extent to which the observed patterns can be generalized to other coffees, origins, roasting conditions, and preparation methods. Furthermore, RSM optimization was performed only using the 80% Arabica/20% Robusta (w/w) ground coffee. The resulting conditions were applied to both blends and their corresponding spent coffee grounds as a common extraction protocol but were not independently optimized for these matrices. Therefore, the optimal extraction conditions may differ between the two coffee blends and between original ground coffee and spent coffee grounds recovered after brewing. Drying the spent coffee grounds at 45 °C for approximately 12 h between brewing cycles may have affected their phenolic content, antioxidant-related activity, and particle morphology. Although the analytical results for solid samples were expressed on a dry-mass basis, the grounds retained measurable residual moisture after drying at 45 °C for approximately 12 h. The differences in residual moisture among the samples may have contributed to variation in their measured properties, while drying-related changes, including possible oxidation of phenolic compounds, cannot be excluded. As the effects of drying were not evaluated independently, their contribution to the observed changes could not be separated from that of successive brewing cycles. The simultaneous variation in blend composition and roast level also prevented their individual effects from being isolated. Future studies should control these factors independently to determine their respective contributions to the observed changes during sequential reuse. The DPPH and Folin–Ciocalteu assays provide overall antioxidant-related responses but do not identify the compounds responsible or establish their biological relevance. Methanol was used exclusively for the analytical extraction of solid samples and was not part of the investigated valorization approach. Consequently, the optimized extraction conditions should not be interpreted as a food-processing protocol or as a procedure for producing an extract intended for consumption. The absence of process-level energy and material balances also limits the assessment of process sustainability. In addition, the qualitative assessment of representative optical microscopy images does not permit quantitative conclusions regarding particle-size distributions or systematic changes in particle morphology across brewing cycles. Particle dimensions were not calibrated in micrometers, and the effects of field selection, suspension concentration, and particle agglomeration were not systematically evaluated. The relatively small voluntary consumer sample also limits the generalizability of the findings regarding consumer acceptance. Accordingly, the findings support the potential for further valorization of the residual material, but do not by themselves establish food safety, economic feasibility, environmental superiority, or market acceptance.

Future research should focus on evaluating the practical potential of sequential spent coffee grounds reuse in Greek coffee preparation. Further studies should investigate the effects of brewing conditions and coffee composition on the properties of the resulting beverages and residual grounds. Detailed chemical characterization could clarify the changes in individual compounds across successive brewing cycles, while safety, sensory quality, and consumer acceptance should be evaluated using defined beverage preparations. Material and energy balances, together with techno-economic and life cycle assessments, would help determine whether sequential reuse offers meaningful environmental and economic benefits. Such investigations would establish whether this approach can progress from an experimental reuse strategy to a practical circular bioeconomy application.

5. Conclusions

This study investigated the sequential brewing of Greek coffee as a potential approach for spent coffee grounds valorization, combining chemical characterization of the original grounds, reused grounds, and resulting beverages with analytical ultrasound-assisted extraction and consumer assessment. The ultrasound-assisted extraction was used exclusively

to prepare the solid samples for chemical analysis and did not constitute a separate valorization process. The optimized conditions for DPPH radical-scavenging capacity were 22% methanol, 61 min, and 47 °C, with a predicted response of approximately 16,614 mg/kg dry weight. Experimental validation yielded 16,039 ± 432 mg/kg dry weight, supporting the predictive performance of the model under the selected conditions. Methanol concentration and extraction time were the principal factors affecting the response, whereas temperature was not statistically significant within the investigated range.

Sequential brewing reduced the total phenolic content of the spent grounds from 13,724.0 ± 330.5 mg/kg dry weight in the original 70% Arabica/30% Robusta blend to 1834.4 ± 42.1 mg/kg after Cycle 1 and 538.4 ± 33.0 mg/kg after Cycle 2. For the 80% Arabica/20% Robusta blend, the corresponding values were 13,604.0 ± 361.7, 3522.4 ± 75.0, and 1418.4 ± 63.2 mg/kg dry weight, respectively. These results demonstrate that measurable phenolic content remained in the grounds after successive brewing cycles. The beverages also retained measurable phenolic content, although it decreased across the cycles.

The consumer questionnaire, completed by 82 participants, provided an exploratory assessment of awareness, attitudes, and willingness to accept applications involving spent coffee grounds. Regarding hypothetical Greek coffee prepared using coffee-residue valorization technologies, 5 participants (6.1%) indicated that they would be willing to consume it, 34 (41.5%) selected "Maybe", and 43 (52.4%) selected "No". With regard to purchasing products derived from coffee-residue valorization, 36 participants (43.9%) assigned an intention score of 4 or 5 on the five-point scale (median = 3, IQR = 1). These findings indicate that consumer acceptance varied according to the application considered and should be interpreted in light of the exploratory nature of the survey.

The novelty of this work lies in the combined investigation of successive Greek coffee brewing, changes in the chemical characteristics of both beverages and residual grounds, extraction optimization, and consumer perceptions of spent coffee grounds reuse. The valorization approach investigated in this study was the sequential reuse of spent coffee grounds as a raw material for further Greek coffee preparation. The study did not investigate the production of antioxidant extracts or other products from the residual grounds. However, the study does not establish industrial-scale feasibility, process-level energy or economic performance, or the safety and acceptability of commercial products. The questionnaire was exploratory and was not presented as a validated psychometric instrument. Further work is required to establish practical performance and application-specific suitability.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su182111368/s1. Online Consumer Questionnaire on Greek Coffee Consumption and Spent Coffee Grounds Valorization.

Author Contributions: Conceptualization, E.P.; methodology, I.C.S. and E.P.; software, I.C.S. and E.P.; formal analysis, I.C.S.; investigation, I.C.S. and E.P.; data curation, I.C.S. and E.P.; writing—original draft preparation, I.C.S. and E.P.; writing—review and editing, E.P.; visualization, I.C.S. and E.P.; supervision, E.P. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of New York College, Greece (NYC23.07.26.B; approved on 23 July 2026).

Informed Consent Statement: Informed consent was obtained electronically from all subjects involved in the questionnaire study. Participation was voluntary, and participants were informed about the purpose of the study, the procedures for data protection, the intended use of the results, and their right to discontinue participation before submission.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request. Restrictions apply to the availability of the survey data due to ethical and confidentiality considerations.

Conflicts of Interest: The authors declare no conflicts of interest.

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